

U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

### Remarks

#### Amendments to the Claims

Claim 10 has been cancelled as failing to limit the claim from which it depends and lacking proper antecedent basis.

#### The Claimed Invention

Applicant is the first to recognize that lipoprotein and/or cholesterol levels affects a female's ability to reproduce. Applicant is the first to recognize that SR-BI, by virtue of its role as the only known transporter of cholesterol, which is critical to steroid production, plays a major role in female reproduction by virtue of its role in steroid production, and can therefore be a basis to inhibit pregnancy and to treat disorders characterized by overproduction of steroids.

Applicant demonstrated the criticality of SR-BI, and its role on lipoprotein and cholesterol levels, using SR-BI knockout mice. The homozygous knockout females are unable to carry a fetus to term. Heterozygotes are able to do so. These studies are described in the patent application as filed.

The data presented in the specification clearly demonstrate that multiple compounds have been identified and are representative of widely disparate species, ranging from nucleic acid molecules encoding SR-BI to organic compounds for lowering cholesterol.

In example 5, Applicant demonstrated that transient increases in SR-BI expression following administration of an adenoviral vector encoding SR-BI results in a decrease in cholesterol levels. In example 6, the Applicant demonstrated that SR-BI knockout animals exhibit the opposite phenotype; increased cholesterol levels (see Table 3). Data in example 7

U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

further shows that these animals are also infertile. Antibody blocking studies have also showed similar results using antibodies to block cholesterol transport, resulting in lowered cholesterol levels, as described in Example 8, page 55.

The reagents and methods provided in the present specification were used to subsequently show the restoration of fertility in an SR-BI knockout mouse (or their transplanted oocytes) in the absence of ovarian and/or extraovarian SR-BI expression by manipulations that modify the structure, composition and/or abundance of their abnormal plasma lipoproteins. These manipulations centered around the administration of probucal, a cholesterol lowering drug (Mcittinen, *et al.*, *J. Clin. Invest.* 108:1717-1722 (2001)).

The application therefore teaches one skilled in the art that SR-BI is essential for normal female fertility; that decreasing levels of SR-BI activity decreases cholesterol levels and alters lipoprotein levels; and that restoring SR-BI activity normalized cholesterol levels and lipoprotein profiles, with a concurrent increase in steroidogenesis and female fertility. The application further teaches that one can use any number of compounds to alter SR-BI levels: viral vectors to increase SR-BI expression; antibodies to block SR-BI activity and concurrent transport of cholesterol; and organic molecules identified by routine screening assays using SR-BI binding and uptake studies. These compounds alter SR-BI activity either by increasing the amount of transport or by decreasing transport (for example, using viral vectors or antibodies).

The present application, and its analysis of SR-BI knockout mice, ties together fertility and cholesterol level. The direct correlation that exists between cholesterol/HDL and the existence of SR-BI, lies at the core of the claimed method. Many compounds that already exist

U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

for regulating cholesterol levels can be used to inhibit fertility (i.e. pregnancy) via the inhibition of SR-BI expression or activity. The compounds can also be used to treat disorders characterized by elevated steroidal levels. It would not require undue experimentation to identify these known compounds, the patients to be treated, or what constitutes an effective amount. Moreover, one skilled in the art would have no difficulty in identifying the scope of the claimed method in view of the specification, the examples, and the knowledge available to those skilled in the art.

**Rejection Under 35 U.S.C. § 112, written description**

Claims 1-10, 12, 15, 16 and 20-22 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention.

***The Legal Standard for Written Description***

"There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed". *Wertheim*, 541 F.2d at 262, 191 USPQ at 96 (CCPA 1976). The written description requirement for a claimed genus may be satisfied through a sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with a known or a disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the Applicant was in possession of the claimed genus (see i)(C), above). See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. On the other hand, there may be a situation where one species adequately supports a genus. See, e.g., *Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326-27.

In the patent context, not all functional descriptions of genetic material necessarily fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if, in the knowledge of the art, the disclosed function is sufficiently correlated to a particular, known structure. (*Amgen v. Hoechst Marion Roussell* 314 F.3d 1313 Fed.Cir. 2003).

***The Specification Complies with the Written Description Requirement***

The claimed invention is based on the clear cut description of the nexus between fertility, steroidal levels and cholesterol levels. The specification is replete with support for this novel connection (see, for example, page 7, lines 21-22; page 13, lines 14-15; page 49, lines 16-20; page 49, lines 21-24 and Example 7). Because of this established connection, the applicant is claiming all compounds that alter lipoprotein, LDL, HDL, or cholesterol levels mediated by SR-BI for the purpose of inhibiting pregnancy or decreasing production of steroids in a mammal. The examples in the specification clearly show that, for example, antibodies raised against a portion of the extracellular domain of the protein inhibit the selective uptake of HDL and delivery of HDL cholesterol to the steroidogenic pathway in cultured adrenal cells (Example 8, and in particular, Table 4, wherein anti-SR-BI inhibited the production of tritiated steroid derived from tritiated HDL). Adenoviral vectors encoding SR-BI (Example 5) are an additional example

U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

of a compound that has been shown to alter cholesterol levels (as will be discussed below in more detail as it relates to enablement). In each of Examples 3, 5 and 8, the applicant has reduced to practice a distinct compound for altering cholesterol levels.

In Example 6, the SR-BI gene was inactivated in embryonic stem cell by standard recombination methods (strategy shown in Example 3). Blastocysts were injected with the embryonic stem cells, producing 24 male chimeric mice. F1 offspring (from crosses between the chimeric mice and wild type females) were either homozygous or heterozygous at the SR-BI locus. F1 intercrosses generated F2 progeny, wherein the males were fertile and the homozygous females (-/- at the SR-BI locus) were unable to produce offspring (Example 7 further discusses the reproductive studies on these mice to make a determination regarding fertility). Example 6 then discusses the resultant elevated levels of plasma cholesterol in which the cholesterol and apolipoprotein profiles of the *heterozygous* mutants were similar to those of wild type controls, except that there was an increase in the amount of cholesterol in the HDL fractions. In the F2 *homozygous* mutant animals (-/- at the SR-BI locus), the cholesterol was found in a large, somewhat heterogeneous peak in the HDL range (*via* FPLC cholesterol analysis). In view of the foregoing results (as discussed in detail in Example 6), one of ordinary skill in the art will readily recognize, not only the direct correlation that exists between cholesterol/HDL and the existence of SR-BI, but also the many compounds that already exist for regulating cholesterol levels. These compounds, well known for altering cholesterol levels, can be used to inhibit pregnancy or decrease steroidal overproduction via the modulation of SR-BI expression or activity. For

U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

further support in this regard, the examiner's attention is again drawn to the attachments to the Amendment filed August 13, 2002.

The legal standard for written description does NOT require that the applicant reduce to practice all of the claimed species that may fall within the claimed genus. In this regard, the Examiner's attention is not only drawn to the three widely disparate types of compounds discussed in the foregoing paragraph, but additionally, and respectfully, drawn to the section in the specification entitled: "I. Inhibitors of SR-BI transport of cholesterol", and the section entitled: "II. Methods of Regulation of SR-BI cholesterol transport to alter steroidogenesis". The description clearly conveys that, in addition to the classes of compounds actually used to show reduction to practice, a number of other molecules were known and could be screened for utility in the claimed method.

The test under 35 U.S.C. 112 was clearly articulated by the Court in *Amgen Inc. v. Hoechst Marion Roussel, Inc. and Transkaryotic Therapies, Inc.* 314 F.3d 1313, 65 USPQ 2d (Fed. Cir. 2003) as being different in the case where, as here, the reagents that could be used in a claimed method were known, and where one was claiming a novel class of compounds. The Court weighed heavily the fact that one was not claiming the class of compounds *per se*, but the use of the compounds. A different degree of description is required where compounds are known and one only needs to provide the criteria for their selection and use - a degree clearly met by applicant.

U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

**Rejection Under 35 U.S.C. § 112, enablement**

Claims 1-10, 12, 15, 16 and 20-22 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled for inhibiting pregnancy in any animal models other than SR-BI knockout female mice.

***The Legal Standard for Enablement***

The Court of Appeals for the Federal Circuit (CAFC) has described the legal standard for enablement under § 112, first paragraph, as whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation. See, e.g., *Amgen v. Hoechst Marion Roussell* 314 F.3d 1313 (Fed. Cir. 2003) and *Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d at 165, 42 USPQ2d at 1004 (quoting *In re Wright*, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). See also *In re Fisher*, 427 F.2d at 839, 166 USPQ at 24; *United States v. Teletronics, Inc.*, 857 F.2d 778 (Fed. Cir. 1988); and *In re Stephens*, 529 F.2d 1343 (CCPA 1976). The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *M.I.T. v. A.B. Fortia*, 774 F.2d 1104 (Fed. Cir. 1985). In addition, as affirmed by the Court in *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524 (Fed. Cir. 1987), a patent need not teach, and preferably omits, what is well known in the art.

Whether the disclosure is enabling is a legal conclusion based upon several underlying factual inquiries. See *In re Wands*, 858 F.2d 731, 735, 736-737, 8 USPQ2d 1400, 1402, 1404 (Fed. Cir. 1988). As set forth in *Wands*, the factors to be considered in determining whether a claimed invention is enabled throughout its scope without undue experimentation include the

U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, and the breadth of the claims. In cases that involve unpredictable factors, "the scope of the enablement obviously varies inversely with the degree of unpredictability of the factors involved." *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation 'must not be unduly extensive.' *In re Atlas Powder Co., v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir.1984).

As noted in *Ex parte Jackson*, the test is not merely quantitative, since a considerable amount of experiment is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. See *Ex parte Jackson*, 217 USPQ 804, 807 (PTO Bd. App. 1982). The adequacy of a specification's description is not necessarily defeated by the need for some experimentation to determine the properties of a claimed product. See *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F3d 956, 965-966 63 USPQ2d 1609, 1614 (Fed. Cir. 2002). There is no requirement for examples.



U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

***The Specification Complies with the Enablement Requirement***

As discussed above, the claims are based on the discovery of *the nexus between fertility, steroidal levels, and cholesterol levels* (see, for example, page 7, lines 21-22; page 13, lines 14-15; page 49, lines 16-20; page 49, lines 21-24 and Example 7). Because of this established connection, the applicant is claiming all compounds that alter lipoprotein, LDL, HDL, or cholesterol levels mediated by SR-BI for the purpose of inhibiting pregnancy or decreasing production of steroids in an individual having a steroidal overproduction disorder. One can readily identify patients to be treated. It should be noted that the class of patients does not overlap with the normal class of patients treated with drugs altering cholesterol and/or lipoprotein levels. Most of these individuals are older - in the case of women, cholesterol does not typically increase until after menopause.

The examples have been discussed above. The paper showing restoration of fertility by administration of a cholesterol lowering drug, probucal, also discussed above, provides further support for the claimed method. The mere fact that this data was obtained after the filing date of the application makes the data no less relevant for demonstrating enablement which is asserted in the application, where the paper uses methods and materials known and readily available at the time the application was filed. The role of SR-BI in fertility is clearly established by the examples in the specification, whether it is to be restored in the case of knockout animals with insufficient SR-BI, or inhibited in the case of administering inhibitors of SR-BI. The role of SR-BI in cholesterol transport was known (see, page 10, lines 19-21).

U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

Because HDL is the only lipoprotein present in substantial amounts in the follicular fluid surrounding the developing oocyte in humans, based on the data in the examples, it is expected that changes in HDL and/or SR-BI in humans may disturb oocyte maturation or function, and thus contribute to infertility. The present application, and its analysis of SR-BI knockout mice, ties together fertility and cholesterol level. The direct correlation that exists between cholesterol/HDL and the existence of SR-BI, lies at the core of the claimed method. Many compounds that already exist for regulating cholesterol levels can be used to inhibit pregnancy, or decrease steroidal production *via*, for example, the modulation of SR-BI expression or activity. It would not require undue experimentation to identify these compounds, the patients to be treated, or what constitutes an effective amount. Moreover, one skilled in the art would have no difficulty in identifying the scope of the claimed method in view of the specification, the examples, and the knowledge available to those skilled in the art.

The following enclosed articles establish that mouse models are accepted models for human gonadotropin signaling pathologies, and thus that the data applicant has provided with respect to the transgenic mice would be expected to be predictive of results in humans.

Burns and Matzuk, "Minireview: Genetic Models for the study of Gonadotropin Actions" *Endocrinology* 143(8):2823-2835 (2002)

Matzuk and Lomb, "Genetic dissection of mammalian fertility pathways" *Nature Cell Biology & Nature Medicine Fertility Supplement* (2002) online table

The applicant respectfully asserts that one of skill in the art would understand from the specification which compounds to use, and how to derive appropriate doses with minimal routine

U.S.S.N. 09/148,012  
Filed: September 4, 1998  
AMENDMENT

experimentation to practice the claimed method and inhibit fertility or treat a disorder characterized by excessive steroidal production.

For the foregoing reasons, Applicant submits that the claims 1-10, 12, 15, 16, and 19-22 are patentable.

Respectfully submitted,



Patrea L. Pabst  
Reg. No. 31,284

Date: January 17, 2006

PABST PATENT GROUP LLP  
400 Colony Square, Suite 1200  
1201 Peachtree Street  
Atlanta, Georgia 30361  
(404) 879-2151  
(404) 879-2160 (Facsimile)

0013-7227/02/0218-0018  
Printed in U.S.A.

Endocrinology 143(2):18-28  
Copyright © 2002 by The Endocrine Society

## Minireview: Genetic Models for the Study of Gonadotropin Actions

KATHLEEN H. BURNS AND MARTIN M. MATZUK

Department of Pathology (K.H.B., M.M.M.), Department of Molecular and Human Genetics (K.H.B., M.M.M.), and Department of Molecular and Cellular Biology (M.M.M.), Baylor College of Medicine, Houston, TEXAS 77030

Fertility in both sexes relies on complex physiological and molecular processes with many levels of regulation, and our ability to alter the mammalian genome using transgenic technology has greatly enhanced our understanding of these processes. There are numerous commonalities in human and mouse physiology, and the list of mouse models recapitulating recognized and idiopathic human reproductive defects is growing at an ever-increasing rate. In this review, we focus on genetic models of gonadotropin actions, summarizing features of transgenic mice that phenocopy defects in gonadotropin production and gonadotropin receptor responses seen in patients. In addition, we provide examples of mouse models with genetic alterations influencing pituitary FSH and LH

production and their effects. These include: 1) transgenic mice with aberrations in steroid hormone, inhibin, and activin feedback pathways; 2) knockouts that demonstrate specific *in vivo* functions of pituitary transcription factors; and 3) models with alterations in other pituitary hormones, IGF-I, and leptin signaling pathways, which affect both the central and peripheral endocrine axis. What we have to learn from these and other models will continue to revise our conceptions of physiology, identify new targets for contraception, and improve our tools for understanding, diagnosing, and treating cases of human endocrinopathies and pathologies of the reproductive tissues. (*Endocrinology* 143: 2823–2835, 2002)

"Whenever man comes up with a better mousetrap, nature immediately comes up with a better mouse." James Carrawell

### Mouse Models and Human Gonadotropin Signaling Pathologies

THE HYPOTHALAMIC-PITUITARY-GONADAL (HPG) axis is fundamental to the endocrine control of gametogenesis in mammals, and because of its long-recognized physiological importance, many human mutations disrupting the normal function of the axis have already been characterized, and several relevant mouse models have been engineered. The pituitary gonadotropins, FSH and LH, are central to this endocrine communication. FSH and LH are heterodimeric glycoproteins each comprised of a common  $\alpha$ -subunit and a unique  $\beta$ -subunit; functional dimers are synthesized and secreted into the circulation in response to hypothalamic GnRH. The human common  $\alpha$ -, FSH $\beta$ -, and LH $\beta$ -subunits are glycosylated 92-, 111-, and 121-amino acid peptides, respectively (1). FSH and LH elicit intracellular signaling pathways by binding to their respective G protein-coupled transmembrane receptors, FSH receptor (FSHR) and LH receptor (LHR), in somatic gonadal cells to regulate fol-

licular development, ovulation, and steroidogenesis in females, and spermatogenesis, testicular growth, and steroidogenesis in males (2,3). The human FSHR and LHR proteins are 695- and 701-amino acid glycoproteins, respectively. Loss-of-function and gain-of-function human mutations in components of the HPG axis have been described previously (4–6), and examples are given in Table 1 and illustrated in Fig. 1.

The pulsatile secretion of FSH and LH is regulated by the decapeptide GnRH, also referred to as LHRH. Hypothalamic production of GnRH and signaling through pituitary GnRH receptor (GNRHR; a 328-amino acid G protein-coupled receptor) appear essential to maintaining serum gonadotropins, and ultimately fertility in mammals. Mutations in genes mediating the developmental migration of GnRH-releasing neurons (as seen in the *KAL* gene causing Kallman's syndrome), or aspects of GnRH processing (as seen in mutations in the *PCT* protein processing enzyme) lead to hypogonadotropic hypogonadism (HHG) (7,8). However, to date, no defined loss-of-function mutations in the GnRH gene itself have been described in patients with HHG (9). Mutations in the GNRHR gene resulting in GnRH resistance have been described in HHG patients who are homozygous or compound heterozygous for missense mutations (6). *In vitro* studies of these mutant GNRHR products indicate that they are hypomorphic mutations that cause deficiencies in GnRH binding or intracellular signal initiation. Clinically, a majority of these patients respond to administration of exogenous GnRH treatment, and in one reported case, pulsatile provision of GnRH was able to induce ovulation and restore a woman's fertility (10).

In contrast to humans, in which no GnRH mutation has been identified, a naturally occurring 33.5-kb deletion truncating the *Gnrh* gene in mice (11) results in a model of he-

Abbreviations: ActRII, Activin receptor type II; CG, chorionic gonadotropin; DAX-1, dosage sensitive sex-reversal-adrenal hypoplasia, congenital critical region on the X chromosome 1, gene 1; Egr, early growth response; ER, estrogen receptor; FSHR, FSH receptor; GHR, GH receptor; GNRHR, GnRH receptor; hCG, human CG; HHG, hypogonadotropic hypogonadism; HPG, hypothalamic pituitary-gonadal; *Inhb*, inhibin  $\alpha$ ; *lacZ*,  $\beta$ -galactosidase; LHR, LH receptor; MMTV, mouse mammary tumor virus; OTC, orthodenticle homolog 1; Pit1, pituitary-specific transcription factor 1; POUH1, POU domain, class 1, transcription factor 1; PRL, prolactin; Prpl, paired like homeodomain factor 1, prophet of Pit1; SF-1, steroidogenic factor-1; *Sry*, sex determining region of chromosome Y; WT-1, Wilms tumor 1.

2823

TABLE 1. Genetic causes of reproductive axis phenotypes in patients

Gene	Clinical presentation (autosomal recessive unless specified)	Reference
GnRH	No ORF mutations or deletions are reported, although deletions at the GnRH locus have been described in patients with HHG	9
GnRH Gln <sup>106</sup> Arg Arg <sup>107</sup> Gln	Hypomorphic alleles; males present with HHG, low serum testosterone, delayed puberty and oligospermia; females have primary amenorrhea, low serum estrone, FSH and LH No activating mutations described	6 138
$\alpha$ -Subunit	No human mutations reported; predicted to result in embryonic lethality because the $\alpha$ -subunit is also shared with hCG in humans	4
FSH $\beta$ Frameshift Val <sup>61</sup> Cys <sup>63</sup> Gly	Loss-of-function mutations; females present with primary amenorrhea and infertility; males with azoospermia, low testosterone and high serum LH	139, 140
LH $\beta$ Glu <sup>60</sup> Arg	Loss of function mutation described in a homozygous, infertile male with low serum testosterone, no Leydig cells, and high levels of immunoreactive LH	28
FSHR Ala <sup>169</sup> Val Asp <sup>607</sup> Gly	Loss-of-function mutation; females have a defect in follicular maturation; males have decreased testes volume and oligospermia Activating mutation described in a hemizygous, hypophysectomized but fertile male	25, 141 142
LHR Ser <sup>610</sup> Tyr Ala <sup>593</sup> Pro	Hypomorphic allele; micropenis More severe loss-of-function mutation; XY individuals develop female external genitalia, intraabdominal testes with no Leydig cells; XX individuals present with amenorrhea	143 144, 145
Asp <sup>578</sup> Gly	Activating mutation causing autosomal dominant familial male precocious puberty; no female phenotype	32

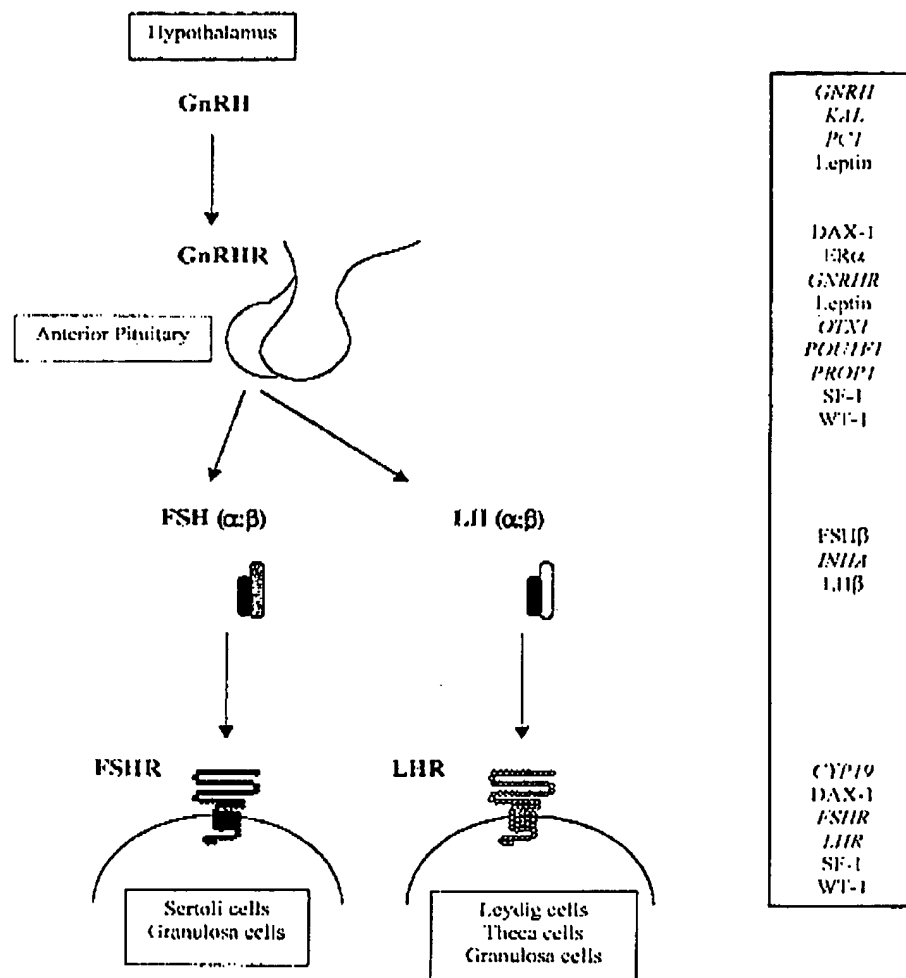
editary hypogonadism (*hpg*), which phenocopies the HHC of patients with defects in GnRH production or responsiveness. Hypogonadism is also a feature of a knockout mouse model harboring a null allele at the glycoprotein hormone common  $\alpha$ -subunit locus; besides reproductive defects, the knockout mouse is hypothyroid and displays proportional dwarfism owing to loss of TSH function (12). These and other relevant mouse models with reproductive phenotypes are shown in Table 2. No mutations altering the amino acid sequence of the common  $\alpha$ -subunit have been described in humans. It has been hypothesized that a deleterious mutation in the human  $\alpha$ -subunit gene (*GLYA*) would result in embryonic lethality because in humans (and not in mice) the  $\alpha$ -subunit is also shared with human chorionic gonadotropin (hCG) (4). Embryo-derived hCG maintains ovarian luteal cells, and is thereby required for pregnancy during the first trimester. Substantiating this, the most highly expressed hCG $\beta$ -subunit-encoding genes are highly conserved, with no individuals homozygous for a deletion of the C $\beta$  cluster (13).

Recently, a second GnRH peptide (GnRH II) (14) and its receptor (type II GnRHR) (15) have been described in several species, including humans. GnRH I (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>) and GnRH II (pGlu-His-Trp-Ser-Ile-Gly-Trp-Tyr-Pro-Gly-NH<sub>2</sub>) differ by three amino acids, their affinities for the two described G protein GnRH receptors, and their tissues of expression and potential action. Human GnRH II is expressed in several regions of the brain, including the amygdala, caudate nucleus, hippocampus and thalamus, as well as outside the central nervous system in the kidney, bone marrow, prostate, endometrium, and ovary (14, 16, 17). Type II GnRHR is expressed ubiqui-

tously and is down-regulated in multiple tumor cell lines, which has led to the suggestion that it may be involved in inhibiting cell proliferation and prompting differentiation programs (15); indeed, *in vitro* experiments indicate that GnRH II has antiproliferative effects on ovarian carcinoma cells (18). Functional characterizations of GnRH II and its receptor *in vivo* have not yet been reported, and the roles of GnRH II signaling, if any, in gonadotropin physiology remain unknown.

Loss of FSH signaling causes infertility in women (19), and this condition can be modeled by disrupting either the *Fshb* (*Fshb*) or *Fshr* loci in mice. Women who are homozygous or compound heterozygous for inactivating ligand (frameshift/truncation and missense) or receptor (missense) mutations exhibit normal preantral follicle development, but no antral-stage follicles capable of ovulation form. These women typically present clinically as cases of primary amenorrhea and sexual infantilism. Both *Fshb* and *Fshr* knockouts in mice phenocopy the human mutations in this regard, displaying female infertility, uterine hypoplasia, and folliculogenesis blocks before antrum formation (20–22). Although estradiol is present in the serum of these mice, the mRNAs encoding P450 side chain cleavage and P450 aromatase estrogenic enzymes are markedly down-regulated in the ovaries of *Fshb* knockout mice (23). Both *Fshb* and *Fshr* knockout females also exhibit high levels of circulating LH (20–22). In both models, this is a progressive effect, with less LH seen in younger mice than in older mice. It is believed that LH and LH-induced androgen synthesis in the *Fshr* model contribute to the development of ovarian sex-cord stromal tumors, which are seen in 92% of these mice by 12 months of age. In these tumors, there is loss of granulosa cell proliferation control

FIG. 1. The HPG endocrine axis is illustrated with several key components of the signaling relay. Within the central portion of the axis, the actions of hypothalamic GnRH mediate the production of the anterior pituitary heterodimeric gonadotropins, FSH and LH. These bind to FSHR and LHR in the periphery to regulate gametogenesis and steroidogenesis. At each level of the axis, mutations in human genes are known to cause defects in the development or function of the reproductive system; examples are listed on the right.



programs, accompanied by an up-regulation of Sertoli cell markers, Müllerian-inhibiting substance, and GATA-4 transcription factor (24). In men, FSH $\beta$  and FSHR mutations have been associated with variable degrees of impairment of spermatogenesis and sometimes with delayed puberty (4). However, men with FSHR null mutations can father children (25). *Fshb* and *Fshr* knockout male mouse models are fertile but have lowered sperm counts and decreased testicular size. Therefore, it seems that FSH activity, while necessary for optimal sexual development and spermatogenesis, is dispensable for male, but not female, fertility in both humans and mice.

A transgenic mouse model overexpressing high levels of human FSH has also been developed (26). The transgenic males demonstrate stimulated Leydig cell function, elevated serum testosterone, and infertility; females exhibit infertility with hemorrhagic and cystic ovaries. These mice may prove valuable in modeling gonadal and more global physiological effects of FSH hyperstimulation. Consistent with the high conservation of FSH $\beta$  genes in mice and humans, synthesis

of human FSH $\beta$  under its own promoter in the gonadotropes of mice lacking the endogenous FSH $\beta$ -subunit restores normal fertility in male and female mice (27). Moreover, partial rescue of the knockout phenotype was observed in mice ectopically expressing human FSH (human  $\alpha$ - and FSH $\beta$ -subunits) under the direction of the mouse metallothionein-1 promoter (27). Together, these studies demonstrate functional conservation between mouse and human FSH $\beta$  promoter elements, as well as between the two FSH $\beta$  peptides in terms of their abilities to assume glycosylation patterns, dimerize with the mouse  $\alpha$ -subunit, route to secretory granules independent of LH, and bind and activate the mouse FSHR.

Loss of isolated LH signaling function can arise from defects in the unique LH $\beta$ -subunit or LHR. One loss-of-function missense mutation in the hormone subunit was identified in an infertile man presenting with low testosterone levels, Leydig cell hypoplasia, and elevated immunoreactive but functionally ineffective LH (28). Several LHR mutations have been discovered to cause defects in receptor

**TABLE 2.** Selected genetic mouse models with reproductive axis phenotypes  
**A.** Models of overexpression and ectopic expression

Construct	Reproductive phenotype	References
mMT promoter and activin/inhibin $\beta$ A sequence	Males with high expression in testes are infertile; seminiferous tubules show patchy lesions and vacuoles	146
Dax1 overexpressor (Dax regulatory region)	XY mice with a weak <i>Sry</i> allele develop as females	90
Bovine glycoprotein hormone- $\alpha$ promoter and diphtheria toxin coding sequence	Pituitary gonadotrophs lost; mice are hypogonadal	84
mMT-1 promoter and human FSH $\alpha$ / $\beta$ subunits	Infertility in both sexes with hyperstimulation of gonadal steroidogenesis in males and hemorrhagic and cystic ovaries in females	26
mMT-1 promoter and follistatin ( <i>Fst</i> ) coding sequence	Progressive infertility in both sexes; males have somatic cell and germ cell defects; females have folliculogenesis defects	79
GII overexpressors (multiple constructs studied)	Compromised reproduction in both sexes	147
Cosmid transgene containing the human CG $\beta$ cluster	Genes are transcribed in the mouse placenta, cerebral cortex, pituitary, and adrenal glands	148
Liver promoter and leptin coding sequence ( <i>skinny</i> mice)	Females have accelerated puberty and late-onset hypothalamic hypogonadism	149
mMT-1 promoter and rat inhibin $\alpha$ coding sequence	Female subfertility; low serum levels of FSH and LH	64
Mifepristone inducible transgene overexpressing inhibin A from liver	Females have a block in folliculogenesis at the early antral stage; males have decreased testis size	150
Bovine glycoprotein $\alpha$ promoter and bLH $\beta$ -hCG fusion	Female infertility, polycystic ovaries, granulosa/theca cell tumors	33
P450 aromatase ( <i>Cyp19</i> ) overexpression (human ubiquitin C and MMTV promoters)	Cryptorchidism and Leydig hyperplasia or tumor development in males	54, 56
Human GnRH promoter and SV40 T antigen sequence	GnRH neurons do not migrate appropriately; mice are hypogonadal	151
Inhibin $\alpha$ subunit promoter and SV40 T antigen sequence	Gonadal tumors and progressive decreases in gonadotropins	152

**B.** Knockout models and naturally occurring loss-of-function mutations

Gene	Reproductive phenotype (in homozygotes unless specified)	References
Activin receptor-type IIA ( <i>Acor2</i> )	Infertility in females and delayed fertility in males; small gonads	76
Activin/inhibin $\beta$ B subunit ( <i>Inhb</i> )	Females are subfertile and litters variably do not survive postnatally	73
Dax1 (DSS-AIC region on the human X)	Males are infertile with progressive degeneration of the germinal epithelium	153
Egr1 (NGF1-A transcription factor)	Female infertility; LH deficiency	94, 95
Early growth response 4 ( <i>Erg4</i> )	Infertility in males; most germ cells undergo apoptosis during pachytene stage	99
Estrogen receptor $\alpha$ ( <i>Ekr</i> ) ( <i>Esr1</i> )	Females are infertile with hemorrhagic ovarian cysts and uterine defects and high circulating LH; males develop disruptions of the seminiferous epithelium	40, 47
Estrogen receptor $\beta$ ( <i>Ekr</i> ) ( <i>Esr2</i> )	Females are subfertile; males are fertile, but develop prostate hyperplasia	154
FSH $\beta$ -subunit ( <i>Fshb</i> )	Female infertility; preantral block in folliculogenesis; males fertile with decreased testis size	20
FSH receptor ( <i>Fshr</i> )	Female infertility; block in folliculogenesis prior to antral formation	21
$\gamma$ -Glutamyl transpeptidase ( <i>Gtpt</i> )	Both males and females are hypogonadal and infertile; phenotype corrected by feeding mice <i>N</i> -acetylcysteine	123
Glycoprotein hormone $\alpha$ -subunit	Males and females are infertile; hypogonadal due to FSH and LH deficiency	12
GnRH ( <i>Gnrh</i> )	<i>hpg</i> mice of both sexes show hypogonadotropic hypogonadism and infertility	11
GII receptor ( <i>Ghr</i> )	Females show delayed puberty and prolonged pregnancy; males have low FSH and testosterone	114, 155
Inhibin $\alpha$ ( <i>Inha</i> )	Female infertility; male secondary infertility; granulosa/Sertoli tumors	60
IGF 1 ( <i>Igf1</i> )	Females are hypogonadal and infertile; impaired antral follicle formation; males are infertile with low testosterone	119
Large tumor suppressor ( <i>Lats1</i> )	Knockout mice develop tumors; pituitary hyperplasia with decreased LH, PRL, and GH levels	156
Leptin ( <i>Lep</i> )	<i>ob/ob</i> mice are obese and infertile with hypogonadotropic hypogonadism in both sexes	124, 125
Leptin receptor ( <i>LepR</i> )	<i>db/db</i> are obese and infertile with hypogonadotropic hypogonadism in both sexes	126
LH Receptor ( <i>Lhr</i> )	Underdeveloped sex organs and infertility in both males and females; spermatogenesis arrested at round spermatid stage; folliculogenesis block prior to antral stage	29, 157
Neuronal Helix-Loop-Helix 2 ( <i>Nhlh2</i> )	Males are infertile and hypogonadal; females are fertile when reared with males	158
Neuronal insulin receptor	Mice exhibit hypothalamic hypogonadism; impaired spermatogenesis and follicle maturation	159

TABLE 2. Continued

Gene	Reproductive phenotype (in homozygotes unless specified)	References
<i>Otx</i>	Prepubescent dwarfism and hypogonadism; progressive recovery of spermatogenesis, follicular development, and fertility	93
P450 aromatase ( <i>Cyp19</i> )	Females are infertile; ovaries do not form CL; mice progressively develop hypergonadotropism and cystic ovaries; males develop progressive infertility with defects in spermatogenesis	50–52
<i>Pit1</i>	<i>Snell</i> dwarf mice have multiple anterior pituitary hormone deficiencies and hypogonadism	160
Prolactin ( <i>Prl</i> )	Females are infertile with irregular estrus cycles	111
Prolactin receptor ( <i>Prlr</i> )	Males infertile; females infertile related to compromised ovulation, fertilization and preimplantation development	112
<i>Prop1</i>	<i>Ames</i> dwarf mice have multiple anterior pituitary hormone deficiencies and hypogonadism	161
Steroidogenic factor-1 (SF-1) ( <i>Nr5a1</i> )	Gonadal and adrenal agenesis in both sexes	80
TSH ( <i>Tshb</i> )	<i>hvt</i> mice are hypothyroid; females show continuous dioestrus, and poor response to gonadotropin-induced superovulation	109
Vitamin D receptor ( <i>Vdr</i> )	Defects in estrogen biosynthesis in males and females; elevated serum gonadotropins	58
Wilms tumor homolog ( <i>Wt1</i> )	Embryonic lethality with gonadal agenesis	162

activity of variable severity. Phenotypes range from micropenis and hypospadias in the case of hypomorphic alleles to male pseudohermaphroditism and female infertility associated with a barrier to preovulatory follicle development, ovulation, and luteinization (4). These conditions are partially modeled by targeted deletion of the *Lhr* locus in mice; homozygotes demonstrate normal prenatal sexual development but are infertile (29). Male *Lhr* knockouts have defects in testicular growth and descent, Leydig cell hypoplasia, and a block in spermatogenesis at the round spermatid stage. Female knockouts have underdeveloped ovaries and uteri, and their ovaries do not contain preovulatory stage follicles or corpora lutea; thus, the *Lhr* knockout in female mice very closely models the pathology of women with LH resistance. It is notable that *hpg* (GnRH mutant) male mice closely phenocopy the *Lhr* mutant male mice, whereas the *hpg* female phenotype mimics that of *Fshb* and *Fshr* knockouts at early timepoints (~6 wk of age) but reflects a combined FSH/LH signaling defect at later points. This observation is consistent with studies showing that androgen administration to *hpg* male mice can restore spermatogenesis (30). Interestingly, the androgens produced by Leydig cells in response to LH may not be as crucial to spermatogenesis as estrogens that are then synthesized from androgen substrates. Provision of exogenous estradiol to *hpg* males increases testis weight and rescues qualitatively normal spermatogenesis in the absence of measurable androgens (31).

Gain-of-function mutations of LHR have been described in families with autosomal dominant male precocious puberty (32). Although no mouse models phenocopy this disorder, transgenics have been engineered to overexpress the bovine LH $\beta$ -subunit with a C-terminal extension of the hCG $\beta$ -subunit (bLH $\beta$ -CTP); there is a prolonged serum half-life of the chimeric LH hormone (33). These *in vivo* studies corroborate findings of Boime and colleagues (34, 35), who demonstrate that the presence of the hCG $\beta$  C terminus of several of the glycoprotein hormone  $\beta$ -subunits (e.g., hCG $\beta$ , FSH $\beta$ ) extends the circulating half-life. Interestingly, female bLH $\beta$ -CTP transgenics have a 10-fold increase in circulating immunoreactive LH and exhibit impaired ovulation, a prolonged luteal phase, ovarian cysts, and granulosa/theca cell

tumors on some genetic backgrounds (36). In addition, in immature transgenic females, enhanced LH and steroid hormones cause precocious follicular development and vaginal opening (37). Studies of these transgenic mice may shed light on the roles of LH in polycystic ovarian syndrome and the development of postmenopausal ovarian stromal tumors, as well as identify genetic modifiers of these phenotypes (36).

#### Mouse Models of Aberrant Steroid Hormone Feedback to the Pituitary

Gonadotropins promote peripheral steroid production by inducing the expression of gonadal steroidogenic enzymes; steroid hormones then produced feedback to negatively regulate pituitary FSH and LH production (1, 38, 39). This paradigm is supported by studies of mouse models with alterations in steroid hormone receptors or steroid biosynthesis that develop secondary anomalies in gonadotropin production. For example, knockout mice lacking an estrogen receptor, *ER $\alpha$* , which is normally expressed in the hypothalamus, pituitary and gonads, exhibit female infertility associated with anovulation and the development of hemorrhagic, polycystic ovaries by 20–22 d of age (40, 41). The disruption to estrogen feedback signaling results in overexpression of gonadotropin subunit mRNAs in the pituitary (42) and chronically elevated serum LH (43). High levels of circulating LH are key to the ovarian pathogenesis in the *ER $\alpha$*  knockout mice, and treatment with a GnRH antagonist precludes ovarian cyst formation (44). In contrast, female mice lacking *ER $\beta$* , which has a relatively restricted pattern of expression, are fertile, and preliminary studies indicate that there is no change in serum LH (45). *ER $\beta$*  signaling plays some role, however, in the regulation of the hypothalamic-pituitary-gonadal endocrine axis, at least in the absence of *ER $\alpha$*  activity. Double knockout females lacking *ER $\alpha$*  and *ER $\beta$*  have higher serum LH levels than do *ER $\alpha$*  single knockouts, and exhibit a different ovarian phenotype in which granulosa cells take on a Sertoli cell-like morphology and express Sertoli cell markers, Müllerian-inhibiting substance, sulfated glycoprotein-2, and *Sox9* (46).

Male *ER $\alpha$*  knockout mice are infertile because of a dis-



2828 Endocrinology, August 2002, 143(8):2823-2835

Burns and Mitzak • Minireview

ruption of fluid reabsorption from the lumen of the epididymis, which is important for concentration and maturation of spermatozoa; increased pressure from the accumulation of fluid leads to testicular atrophy and degeneration of the seminiferous epithelium in mature males (47). This phenotype does not have an obvious relationship to the only clinical case of an ER mutation, that of a man with estrogen resistance caused by a homozygous nonsense mutation in ER $\alpha$  (48). The 28-yr-old patient presented with incomplete epiphyseal closure and continued linear growth into adulthood. Biochemical analyses provided evidence of increased bone turnover, and bone mineral density was low. Serum estradiol, FSH, and LH levels were elevated; serum testosterone was normal. Interestingly, the patient also had impaired glucose tolerance and hyperinsulinemia, and ER $\alpha$  knockout male mice have increases in total body fat and in serum cholesterol and leptin levels after sexual maturity (49). These findings underscore the importance of estrogens in the bone and lipid metabolism, and have important implications for the use of hormonal contraceptives and estrogen replacement therapies in women, and appreciating the effects of exposure to environmental estrogens.

Estrogen production is abrogated in the P450 aromatase knockout mouse model, the female reproductive phenotype of which closely resembles that of ER $\alpha$  knockout mice. Female P450 aromatase knockouts have high serum levels of LH and FSH, are infertile owing to a block in follicular development and ovulation defects, and develop hemorrhagic ovarian cysts by 21–23 wk of age (50, 51). Male P450 aromatase knockouts develop progressive infertility characterized by an arrest in early spermiogenesis, germ cell apoptosis, high circulating LH, and Leydig cell hyperplasia (52). Heritable aromatase deficiency has been described in patients with mutations at the aromatase locus (*CYP17*) (53). In the absence of fetal aromatase (and therefore placental estrogens), there is an elevation of testosterone and androstenedione in both maternal and fetal circulations. This causes pseudohermaphroditism in homozygote females, and virilization of the mother during her pregnancy. In the absence of ovarian aromatase activity, young girls exhibit hypergonadotropic hypogonadism and develop follicular cysts; with the onset of puberty, the ovaries become enlarged and further polycystic and there is progressive virilization. In males, the aromatase deficiency is associated with macroorchidism, high circulating FSH, LH, and testosterone, as well as hyperinsulinemia and abnormal plasma lipids. In both women and men, aromatase deficiency is associated with delayed epiphyseal fusion and osteopenia (53).

An aromatase-overexpressing mouse model has been developed to study the effects of enhanced conversion of androgens to estrogens on male reproduction. These mice carry a transgene expressing human aromatase under the constitutive human ubiquitin C promoter, which is active in multiple mouse tissues by embryonic d 15. These mice have cryptorchidism with Leydig cell hyperplasia and disrupted spermatogenesis, as well as underdevelopment and defects of accessory sex organs. Serum hormone assays reveal elevated estrogen, reduced testosterone, and reduced FSH, with no change in average LH levels but a reduction in LH level variation (54). The cryptorchidism is likely due to a disruption

of steroid effects during prenatal development. The etiology of the Leydig cell hyperplasia is less clear, though both cryptorchidism and exposure to estrogens have been associated with testicular tumorigenesis in mice, and cryptorchidism is a risk factor in humans (55). Leydig cell tumorigenesis is reported in a second aromatase-overexpressing transgenic model in which the aromatase coding sequence is expressed under the mouse mammary tumor virus (MMTV) promoter. The expression leads to Leydig cell tumors that express high levels of ER $\alpha$ , and up-regulation of a G1→S phase cell cycle promoter, cyclin D1, known to be modulated by estrogen exposure (56). The MMTV promoter in this mouse model also drives aromatase expression in the mammary glands and this results in preneoplastic lesions (57). Interestingly, vitamin D has also been recently shown to be important for estrogen biosynthesis in both the ovary and testis. Studies of knockout mice lacking the vitamin D receptor reveal attenuation of gonadal P450 aromatase activity, histological abnormalities of the uterus, ovary, and testis consistent with estrogen deficiency, and elevated levels of LH and FSH (58). Together, the phenotypes of these models indicate important *in vivo* roles for the sex steroid hormones as mediators of the HPG endocrine axis and also as cell cycle controllers and differentiation factors in hormonally responsive peripheral tissues in both males and females.

#### Peptide Hormone Feedback to the Pituitary

In addition to steroid hormones, peptide endocrine factors released from the gonads affect pituitary FSH and LH production. Inhibins ( $\alpha$ : $\beta$ A and  $\alpha$ : $\beta$ B heterodimers) and activins ( $\beta$ A: $\beta$ A and  $\beta$ B: $\beta$ B homodimers, and  $\beta$ A: $\beta$ B heterodimers) are members of the TGF $\beta$  superfamily named for their respective abilities to suppress and enhance FSH production. These peptides are synthesized in granulosa cells of the ovary and Sertoli cells of the testis and are also found in other tissues where they have been implicated in diverse biological processes (59). Male and female knockout mice lacking the  $\alpha$ -subunit (*Inha*<sup>-/-</sup>), and therefore depleted of the biological effects of both inhibins, have high circulating activins and FSH, and develop steroidogenic sex-cord stromal tumors of the granulosa cell and Sertoli cell lineage. These tumors are associated with a cachexia-like wasting syndrome, which typically causes death between 8 and 18 wk of age (60, 61). In addition, superovulation experiments in younger knockout females indicate that there are defects in late stages of follicle development, and transplant experiments demonstrate important paracrine roles of ovarian inhibins in maintaining the granulosa cell phenotype and averting the formation of Sertoli tubule-like structures (62). FSH and LH are critical to the processes of tumorigenesis in these mice, as double mutant mice homozygous for the hypogonadal (*hpg*) mutation at the *Gnrh* locus and the *Inha* knockout allele do not develop tumors (63), and *Fshb* and *Inha* double knockouts develop tumors with later onset and a less aggressive course, a finding that is particularly pronounced in double knockout males (26). In contrast to the *Inha*<sup>-/-</sup> model, mice that overexpress the rat inhibin  $\alpha$  coding sequence under the control of the metallothionein promoter have reduced FSH levels in both sexes and elevated LH levels in females. In addition,

females exhibit subfertility owing to a decrease in the number of ovulated oocytes, a defect that can be corrected with the provision of exogenous gonadotropins (64).

Recent clinical evidence indicates that inhibins may regulate human gonadotropin production, and ultimately affect the duration of a woman's reproductive potential. A point mutation in the human *INHb* gene, resulting in a nonconservative amino acid change (Ala<sup>257</sup>Thr), has been associated with premature ovarian failure (65). The mutation was found in three patients who presented with secondary amenorrhea, low serum estrogens, and elevated gonadotropins, at ages 16, 20, and 24 yr of age. The authors of the study suggest that a decrease in the bioactivity of the mutant inhibin led to persistently enhanced gonadotropins, and premature depletion of ovarian follicular reserves. Low levels of circulating inhibins and high levels of FSH have been associated with premature ovarian failure before, though this study is the first to implicate inhibins in the condition's etiology. We anticipate that future studies of inhibin mutations, and clinical assessments of inhibin and FSH levels preceding ovarian failure, will be informative.

When inhibin  $\alpha$  knockout mice are castrated, they develop steroidogenic tumors of the adrenal cortex, indicating that inhibin signaling is key to proliferation control in the adrenal glands, as well as the gonads (61). Gonadal tumors and the development of adrenal cortical tumors upon castration are also features of a transgenic model expressing the SV40 T antigen (Tag) under the control of the mouse inhibin  $\alpha$  promoter (66, 67). Despite similarities in their phenotypes, the endocrine environments in which gonadal tumors develop in these two mouse models are quite distinct, with a progressive decrease in serum LH and FSH in the Tag overexpressor being noted as ovarian tumorigenesis proceeds (68). Nevertheless, there is a critical function of the gonadotropin hormones in this model, as is demonstrated by the findings that: 1) the *hpg* mutation prevents the Tag mice from developing both gonadal and adrenal tumors (69); 2) adrenal tumor cells express LH-R and respond to LH by up-regulating steroid production *in vitro* (70); and 3) suppression of serum LH by exogenous testosterone administration precludes tumor development (69). Similarly, elevated serum LH in the *blh1 $\beta$ -CTP* transgenic model promotes LH-R expression and induces steroidogenesis in the adrenal cortex (71). Such LH action may also be involved in tumorigenesis in the inhibin  $\alpha$  knockout models, where there is a striking induction of LH-R and P450 aromatase mRNAs in adrenal tumors (our unpublished data). Together, these mouse models provide evidence for important regulatory roles of inhibins and LH on nongonadal steroidogenesis and tumorigenesis.

Homozygote mice with a null mutation engineered at the activin/inhibin  $\beta A$  locus (*Inhib $\beta A$* <sup>-/-</sup>) die neonatally due to craniofacial defects that prevent suckling, and until more recently this had precluded further studies pertaining to the role of activin subunit in reproduction. The null phenotype, however, can be rescued by replacing the activin/inhibin  $\beta A$  coding sequence with that of the activin/inhibin  $\beta B$  gene, conferring the activin/inhibin  $\beta A$  expression pattern on this related sequence (63% amino acid identity). Knock-in mice demonstrate enlarged external genitalia, hypogonadism, and diminished female fertility, indicating unique and previ-

ously unrecognized functions of the activin/inhibin  $\beta A$  protein product in reproduction (72). Serum FSH levels in these mice are slightly increased, though whether this reflects loss of pituitary inhibin  $\alpha/\beta A$  signaling, enhanced activin  $\beta B/\beta B$  signaling, or is simply secondary to gonadal defects remains to be investigated. Knockout mice lacking a functional  $\beta B$  locus (*Inhib $\beta B$* <sup>-/-</sup>) have developmental defects in eyelid closure, prolonged gestation, and a failure of mothers to nurse their litters (62, 73). The latter is also a primary characteristic of oxytocin knockout mice (74), and therefore, together these phenotypes substantiate that activin  $\beta B$  is an important in the induction of this hypothalamic/posterior pituitary hormone (75).

Signaling pathways that mediate activin and inhibin effects are complex, and no receptor or downstream signaling protein mutations have been described that phenocopy the mutant models lacking functional ligands. In the case of activin signaling, however, transgenic mouse models with altered expression of activin-interacting proteins have elucidated specific aspects of activin function in FSH regulation. Activin receptor type II (*ActRII*) has been shown to relay activin-mediated induction of FSH, as knockout mice lacking *ActRII* have suppressed FSH levels in the pituitary and serum; LH levels are not affected. Mutant *ActRII* mice also exhibit gonadal pathologies at least in part due to the lack of FSH, including a delay in fertility and reduced testicular growth in males, and infertility, underdeveloped uteri, follicular atresia and reduced numbers of corpora lutea in females (76). Bioactivities of activin dimers are modulated by their association with a binding protein, follistatin; the interaction is believed to antagonize activin functions in the pituitary (62, 77). Follistatin knockout mice have numerous embryonic defects and die in the perinatal period (78), but the role of follistatin in controlling FSH levels can be appreciated from studies of transgenics overexpressing follistatin under the control of the metallothionein promoter. Lines of mice with widespread expression of the transgene exhibit suppression of serum FSH, and defects in gonadal growth and gametogenesis that can be partially ascribed to FSH deficiency (79).

### Transcriptional Control of the Gonadotropin Genes

Knockout mouse models have led to the functional characterization *in vivo* of several pituitary transcription factors important in mediating gonadotropin expression or providing for the survival of neuroendocrine cell populations in the hypothalamus and pituitary. One of these factors is steroidogenic factor-1 (SF-1), originally identified because of its role in directing the expression of cytochrome P450 steroid hydroxylases in the ovary, testis and adrenal gland. Knockout mice lacking SF-1 have profound defects in endocrine development that affect multiple levels of the HPG axis. Newborn SF-1 knockout mice have gonadal and adrenal agenesis, female sex traits, absence of the ventromedial hypothalamic nucleus, and impaired gonadotrope expression of GnRH-R, FSH, and LH (80–82). Heterozygote mice are phenotypically normal. To circumvent the complexity of the SF-1 null phenotype and establish its role specifically in pituitary hormone production, tissue-specific SF-1 knockout

2830 Endocrinology, August 2002, 143(8):2823-2835

Burns and Matzuk • Minireview

mice have been engineered (83). In these mice, a portion of the endogenous SF-1 locus is marked for excision by Cre-recombinase enzyme by the introduction of tandem *loxP* sites, and a Cre-recombinase-encoding transgene is expressed under the  $\alpha$ -glycoprotein hormone subunit promoter specifically in cells of the anterior pituitary. In both males and females, the pituitary SF-1 loss-of-function caused sexual infantilism and hypogonadism, and gonadotrope cells failed to express appreciable levels of GnRH, FSH, or LH (83). Essentially, all gonadotropic effects of the anterior pituitary are lost in these mice, the hypogonadism being as pronounced as when gonadotrope cells are ablated by targeted toxin expression (84), but there is no defect in gonadal or adrenal development during embryogenesis. In several respects, the SF-1 null mouse phenocopies the effects of a heterozygous mutation in the human *ITZ1* gene encoding SF-1. The mutation has been described in a single patient (85) and is a loss-of-function 2-bp missense change affecting the DNA binding domain of the SF-1 protein and eliminating its recognition of the SF-1 canonical nucleotide sequence. The XY female patient presented with adrenal failure in the first 2 wk of life and had streak gonads with poorly differentiated tubule structures. Despite similarities to the knockout model, there was elevation of gonadotropin hormones in this patient in response to administration of GnRH. It is possible that FSH and LH production during this test relied upon the function of SF-1 transcribed from the normal allele.

The reason for the SF-1 haploinsufficiency seen in humans and not mice remains unclear, though dosage sensitivity is also a hallmark of two other human conditions involving related transcriptional regulators and their roles in gonadal development and sex determination. WT-1 (Wilms tumor-1) and DAX-1 (dosage-sensitive sex-reversal-adrenal hypoplasia, congenital critical region on the X chromosome 1, gene 1) proteins interact directly with SF-1 to promote and repress, respectively, its transactivation of target genes, including Mullerian-inhibiting substance (86). Heterozygote XY children with a dominant negative mutation of WT-1 have Denys-Drash syndrome, characterized by male pseudohermaphroditism, urogenital aberrations, and nephroblastoma (87). In contrast, XY sex reversal can result from duplication of the DAX-1 locus in the dosage-sensitive sex reversal region of the X chromosome (88). Aspects of the Denys-Drash syndrome phenotype are recapitulated in heterozygous mice with a truncation in WT-1 (89), whereas DAX-1 overexpressing transgenics create a dosage-sensitive sex reversal-like condition in mice with a hypomorphic *Sry* (sex determining region of chromosome Y) allele (90). These and other mouse models with sex determination phenotypes are reviewed by Whitworth and Behringer (91).

At birth and throughout adult life, a homeobox-containing transcription factor, OTX1 (orthodenticle homolog 1), is expressed in the pituitary and is involved in the transactivation of several glycoprotein hormone subunit genes. In addition to neurological abnormalities, *Otx*<sup>-/-</sup> mice exhibit a prepubescent period of dwarfism and hypogonadism owing to decreases in GH, FSH, and LH; the condition corrects itself by 4 months of age and knockout mice then have restored growth and gonadal function (92, 93). The defect in these mice does not include alterations in the hypothalamic ex-

pression of GnRH or the pituitary expression of GnRH. During the hypogonadotropic, hypogonadal period, knockout males had a block in spermatogenesis and females had ovaries devoid of antral follicles and corpora lutea. These histological findings were not seen in older fertile knockouts, in which there was recovery of all stages of sperm and follicle development (93). The *Otx* knockout phenotype is particularly intriguing because of the window in which it is apparent, this being the first example of a mouse model for investigating causes and effects of delayed growth and puberty, and the regulation of temporal-restricted molecular mechanisms in the onset of sexual maturity.

Mutant mouse models have defined *in vivo* functions of the early growth response (*Egr*) family of zinc finger transcriptional activators in regulating pituitary hormone production, hindbrain development, peripheral nerve myelination, muscle spindle morphogenesis, and spermatogenesis (94-99). Two of these transcription factors are critical in the establishment of the HPG endocrine axis, EGR1 (also known as nerve growth factor 1A) and EGR4. Though the *Egr1* gene is expressed widely during development, the phenotypes of *Egr1* mutant mice are relatively restricted. The first *Egr1* mutant mouse model was engineered by inserting a neomycin selection cassette into the DNA-binding region of the EGR1 coding sequence (*Egr1*<sup>neo</sup>), and the primary defect is female sterility due to LH insufficiency (94). Homozygote mutant females show loss of estrous cyclicity, uterine hypoplasia, and no luteinized cells in their ovaries, though corpora lutea are evident upon pregnant mare's serum gonadotropin and hCG treatment. FSH is produced in these mice and is up-regulated upon ovariectomy, but LH $\beta$ -subunit mRNA expression is critically compromised. By contrast, *Egr1*<sup>neo</sup> homozygote mutant male mice have no defects in fertility or spermatogenesis (94), though knockout males lacking EGR4 are infertile because of germ cell maturation defects (99). To examine functional redundancies between EGR1 and EGR4, *Egr1*<sup>neo</sup>, *Egr4*<sup>-/-</sup> double mutant mice were bred. Interestingly, double mutant males, unlike *Egr1*<sup>neo</sup> or *Egr4*<sup>-/-</sup> single mutants, demonstrated low levels of serum LH, low serum testosterone, and atrophy of androgen-responsive organs (100). Because steroidogenesis was restored by the addition of an LH receptor agonist, the defect in these mice appears to be in pituitary production of this gonadotropin (100). Therefore, though EGR1 is critical for LH production in the maintenance of female fertility, in *Egr1*<sup>neo</sup> mutant males EGR4 compensates by establishing adequate LH to support androgen production. A second *Egr1* mutant mouse line has been generated by targeted insertion of the *lacZ* ( $\beta$ -galactosidase) gene into the *Egr1* locus (*Egr1*<sup>lacZ</sup>), and these mice have several notable phenotypic differences compared with *Egr1*<sup>neo</sup> mice (95). In these mice, somatotrope development and production of GH in both sexes is impaired, male fertility is critically compromised (but can be rescued by LH administration), and female infertility cannot be rescued by LH administration and is associated with a down-regulation of LH. These findings suggest singular and previously unexpected roles for EGR1 and its target genes in nongonadotrope pituitary cells, LH production in males, and LH production in females. Whether the *Egr1*<sup>neo</sup> allele is a hypomorphic allele or whether the *Egr1*<sup>lacZ</sup> allele

creates aberrations apart from abrogating EGR1 expression remains to be explored.

### Other Pituitary Hormones, IGF-1, and Leptin Affect Multiple Levels of the Endocrine Axis

The physiological complexities of the HPG axis can be further appreciated by studies of mouse models with disruptions of endocrine and paracrine factors known to affect central and peripheral components of the axis. Nongonadotrope anterior pituitary cell lineages and their product hormones may play important roles in establishing bidirectional gonadotrope-gonad communications. *Ames* and *Snell* dwarf mice [with mutations in *Prop1* (paired like homeodomain factor 1; prophet of Pit1) and *Pit1* (pituitary specific transcription factor 1) genes, respectively, encoding pituitary transcription factors] have TSH, prolactin (PRL), and GH deficiencies due to defects in thyrotrope, lactotrope, and somatotrope transcriptional regulation. In addition, both mouse models also display HHG, despite the absence of a direct role for the disrupted transcription factors in gonadotrope ontogeny or gene regulation (101–103). Interestingly, HHG and failure to respond to exogenous GnRH is a feature of patients with homozygous or compound heterozygous *PROT1* mutations (with considerable variability depending upon the exact nature of the mutation), but not in patients with even complete loss-of-function mutations of the human *PIT1* gene, *POU1F1* (POU domain, class 1, transcription factor 1) (104).

To gain an understanding of the direct and indirect effects of nongonadotropin pituitary hormones on ovarian function represents an important challenge today for endocrinologists and researchers. Thyroid hormone has been implicated in the function of the lactotrope and somatotrope cell lineages in the anterior pituitary, which produce PRL and GH, respectively (105). Clinically, both hypothyroidism and hyperthyroidism in women have been associated with menstrual abnormalities, infertility, and complications in pregnancy (106–108). Moreover, analyses of a genetic mouse model of hypothyroidism (*hyt*) caused by TSH deficiency have demonstrated that thyroid hormone is important for peripheral gonadotropin hormone response and female fertility in mature animals (109). Postpartum hyperprolactinemia suppresses the HPG axis by inhibiting GnRH production (110), and defects in PRL regulation have been associated with reproductive abnormalities in patients, though the roles of PRL signaling outside of mammary tissue are still not well understood. Female knockout mouse models lacking PRL or PRL receptor are infertile due to defects in luteinization, and PRL receptor knockouts also exhibit irregularities in estrous cyclicity, as well as a failure to support oviductal embryogenesis (111, 112).

GH is crucial for growth and the onset of sexual maturity, both by its direct effects binding the GH receptor (GHR) and by the effects of downstream IGF-1. Knockout mice lacking the GHR have growth and reproductive defects. Females exhibit subfertility, and a delay in the onset of sexual maturity that can be corrected by administration of IGF-1 (113). GHR knockout males have low levels of circulating FSH and IGF-1, low plasma testosterone, and an attenuation in their

steroidogenic response to exogenous LH owing to down-regulation of LH-R in the testes (114). In contrast, transgenic males that overexpress a human GH transgene under the metallothionein promoter have enhanced transcription of FSH and LH mRNAs, increased serum levels of LH, and an increase in LH release in response to GnRH (103). Female transgenics in which bovine GH expressed by the phosphoenol pyruvate carboxykinase promoter, though unable to maintain pregnancies due to PRL deficiencies, have an increase in the numbers of preovulatory follicles and corpora lutea, and a decreased granulosa cell apoptosis in developing follicles (115, 116). This latter phenotype may in part reflect enhanced IGF-1 function in developing follicles (117). There is no readily evident clinical correlate for these phenotypes, and reports of reproductive defects in patients with alterations in GH regulation most commonly describe cases of hypogonadism in patients with acromegaly. GH overexpression in such patients has been associated with amenorrhea in women and testosterone deficiency in men, even in the absence of hyperprolactinemia (118).

Many biological effects of GH have been attributed to its induction of IGF-1 in peripheral tissues, and studies of *Igf1* knockouts have revealed important functions of this growth factor in prenatal and postnatal growth, as well as within the gonads. *Igf1* knockout males and females are infertile. In males, there is a marked reduction of plasma testosterone, and an inhibition of LH-mediated testosterone production in testicular organ culture experiments (119). In females, there are hypoplastic uteri and no ovulatory response to exogenous gonadotropins (119). Further examination of the *Igf1* null ovaries revealed a block in follicular development reminiscent of that of the FSH knockout mouse and a lack of FSH-R expression; this finding and the coexpression of *Igf1* and *Fshr* mRNAs in healthy, growing follicles has led to the proposal that IGF-1 up-regulates FSH-R and is needed for FSH induction of steroidogenesis (120). Consistent with a role for IGF-1 in steroidogenesis, both girls and boys with Laron syndrome (primary GH resistance) treated with IGF-1 develop elevated serum androgen levels and secondary effects of androgens (121, 122). Therefore, it seems that GH may influence gonadal functioning both by promoting central FSH and LH production, and by enabling gonadotropin response in the gonads, directly and through up-regulation of IGF-1. It should be noted that other physiological parameters influence IGF-1 levels and activities; metabolic defects in  $\gamma$ -glutamyl transpeptidase mice lead to decreases in IGF-1 levels and similar reproductive phenotypes (123).

Just as GH has pleiotropic effects on the HPG axis, leptin, a protein released from adipocytes, acts upon endocrine circuits at multiple locations. Leptin-deficient *ob/ob* mice are obese, infertile, and have characteristics of HHG with enhanced functioning of negative feedback pathways on gonadotropin production reminiscent of immature animals (124, 125). Similarly, leptin receptor-deficient *db/db* mice are obese and hypogonadal (126). *In vitro* studies have indicated that leptin normally functions at both the level of the hypothalamus (enhancing GnRH production), and at the level of the anterior pituitary (enhancing FSH and LH production) to promote the establishment of adult circulating gonadotropin levels (127). Interestingly, leptin may function dichoto-

2832 Endocrinology, August 2002, 143(8):2823-2835

Burns and Matzuk • Mini-review

mously in HPG axis functioning, up-regulating GnRH, FSH, and LH production centrally, but down-regulating steroidogenesis in peripheral tissues (128–131). To understand the effects of leptin and body fat content on the reproductive endocrine axis in humans represents a field of research with important implications for health care practices. Lean persons have decreased circulating leptin levels compared with obese persons (132). It may be speculated that low leptin levels contribute to the suppressed GnRH, low gonadotropins, abnormal glycosylation of gonadotropins, and delay of menarche or amenorrhea observed in young women athletes and anorexics (133, 134). Obese patients have elevated serum leptin levels but may exhibit leptin resistance so that some biological effects of leptin are attenuated. Roles that leptin signaling and other metabolic regulation pathways play in the development of the altered sex steroid profile and amenorrhea seen in obese women remain to be elucidated. Human mutations resulting in obesity have been described affecting both leptin (135, 136) and the leptin receptor (137). In the case of the leptin receptor mutation, two sisters homozygous for a nonsense mutation presented with early-onset obesity, attenuated levels of growth hormone and IGF-I, hypothalamic hypothyroidism, and did not undergo pubertal development. Their endocrine profile showed low levels of estrogens and gonadotropins that persisted without response to GnRH administration. This clinical case underscores the importance of metabolic pathways in the control of the reproductive axis and other endocrine communications, and the continued utility of the *ob/ob* and *db/db* mouse models to study leptin pathologies in humans.

### Conclusions

Cases of human disease and transgenic mouse models offer powerful means for appreciating the molecular components of endocrine pathways, including the direct, indirect and compensatory results *in vivo* of their aberrant functioning. Loss-of-function mutations produce hypomorphic and null alleles in mice that recreate deficiency and resistance syndromes in humans. Transgenic overexpressors and targeted subtle mutations may mimic activating mutations or the effects of hypersecretion syndromes in patients. In some instances, mutant mice closely phenocopy human disorders, stand as important proofs-of-principle, and provide us with models for testing our understanding of etiologies and potential medicinal interventions. In some, unexpected phenotypes call our attentions to unrecognized endocrine relationships, and the pathophysiological bases for previously inexplicable clinical observations. These genetic models will continue to reveal to us aspects of the human endocrine system.

### Acknowledgments

Received January 16, 2002. Accepted March 14, 2002.

Address all correspondence and requests for reprints to: Martin M. Matzuk, M.D., Ph.D., Professor and Stuart A. Wallace Chair, Department of Pathology, One Baylor Plaza, Baylor College of Medicine, Houston, Texas 77030. E-mail: mmatzuk@bcm.tmc.edu.

### References

1. Housfield GR, Perry WM, Ward DN 1994 Gonadotropins: chemistry and biosynthesis. In: Knobil E, Neill JJ, eds. *The physiology of reproduction*. New York: Raven Press, Ltd.; 17:11–1792.
2. Greenwald GS, Roy SK 1994 Follicular development and its control. In: Knobil E, Neill JJ, eds. *The physiology of reproduction*. 2nd ed. New York: Raven Press, Ltd.; vol 1:629–724.
3. Sharpe RM 1994 Regulation of spermatogenesis. In: Knobil E, Neill JJ, eds. *The physiology of reproduction*. New York: Raven Press, Ltd.; vol 1:1363–1434.
4. Themmen APN, Huhtaniemi IT 2000 Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary gonadal function. *Endocr Rev* 21:551–583.
5. Achermann JC, Weiss J, Lee EJ, Jameson JL 2001 Inherited disorders of the gonadotropin hormones. *Mol Cell Endocrinol* 179:89–96.
6. de Roux N, Milgrom E 2001 Inherited disorders of GnRH and gonadotropin receptors. *Mol Cell Endocrinol* 179:83–87.
7. Franco B, Gualdi S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, Carruzzo R, Maestrini E, Pieretti M, Tallon-Miller P, Brown CE, Willard FL, Lawrence CB, Persico GM, Camarino G, Ballabio A 1991 A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path finding molecules. *Nature* 353:529–536.
8. Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders J, Montague CT, Hutton JC, O'Rahilly S 1997 Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet* 16:303–306.
9. Weiss J, Adams L, Whitcomb RW, Crowley Jr WF, Jameson JL 1991 Normal sequence of the gonadotropin-releasing hormone gene in patients with idiopathic hypogonadotropic hypogonadism. *Biol Reprod* 45:743–747.
10. Seminara SB, Beranova M, Oliveira LM, Martin KA, Crowley Jr WF, Hall JE 2000 Successful use of pulsatile gonadotropin-releasing hormone (GnRH) for ovulation induction and pregnancy in a patient with GnRH receptor mutations. *J Clin Endocrinol Metab* 85:556–562.
11. Mason AJ, Hayflick JS, Zoeller RT, Young WS, Phillips HS, Nikolic K, Seeburg PH 1986 A deletion truncating the gonadotropin-releasing hormone gene is responsible for hypogonadism in the hpg mouse. *Science* 231:1366–1371.
12. Kendall SK, Samuelson LC, Saunders TL, Wood RI, Camper SA 1995 Targeted disruption of the pituitary glycoprotein hormone  $\alpha$ -subunit produces hypogonadal and hypothyroid mice. *Genes Dev* 9:7077–7091.
13. Miller-Landholm AK, Bedows E, Bartels CF, Ramey J, MacLennan V, Ruddle RW 1999 A naturally occurring genetic variant in the human chorionic gonadotropin- $\beta$  gene 5 is assembly inefficient. *Endocrinology* 140:3496–3506.
14. White RB, Eisen JA, Kastan TL, Fernald RD 1998 Second gene for gonadotropin-releasing hormone in humans. *Proc Natl Acad Sci USA* 95:3025–3029.
15. Neill JD, Duck LW, Sellers JC, Musgrave LC 2001 A gonadotropin-releasing hormone (GnRH) receptor specific for GnRH III in primates. *Biochem Biophys Res Commun* 282:1012–1018.
16. Cheon KW, Lee HS, Farhat IS, Kang IS 2001 Expression of the second isoform of gonadotropin-releasing hormone (GnRH-II) in human endometrium throughout the menstrual cycle. *Mol Hum Reprod* 7:447–452.
17. Kang SK, Tai CJ, Nathwani PS, Leung PC 2001 Differential regulation of two forms of gonadotropin-releasing hormone messenger ribonucleic acid in human granulosa-luteal cells. *Endocrinology* 142:182–192.
18. Choi KC, Auteursperg N, Leung PC 2001 Expression and antiproliferative effect of a second form of gonadotropin-releasing hormone in normal and neoplastic ovarian surface epithelial cells. *J Clin Endocrinol Metab* 86:5075–5078.
19. Layman LC, McDonough PG 2000 Mutations of follicle stimulating hormone- $\beta$  and its receptor in human and mouse: genotype/phenotype. *Mol Cell Endocrinol* 161:9–17.
20. Kumar TR, Wang Y, Lu N, Matzuk MM 1997 Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. *Nat Genet* 15:201–204.
21. Dierich A, Sairam MR, Monaco L, Fimia GM, Gansmuller A, LeMeur M, Sassone-Corsi P 1998 Impaired follicle stimulating hormone (FSH) signaling *in vivo* targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. *Proc Natl Acad Sci USA* 95:13612–13617.
22. Abel MH, Wootton AN, Wilkins V, Huhtaniemi I, Knight PG, Charlton HM 2000 The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction. *Endocrinology* 141:1795–1803.
23. Burns KJ, Yan C, Kumar TR, Matzuk MM 2001 Analysis of ovarian gene expression in follicle-stimulating hormone  $\beta$  knockout mice. *Endocrinology* 142:2742–2751.
24. Danilovich N, Roy I, Sairam MR 2001 Ovarian pathology and high incidence of sex cord tumors in folliculin receptor knockout (FORKO) mice. *Endocrinology* 147:3673–3684.
25. Tapanainen JS, Aittomaki K, Min J, Vaskivuo T, Huhtaniemi IT 1997 Men homozygous for an inactivating mutation of the follicle stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. *Nat Genet* 15:205–206.
26. Kumar TR, Palapattu G, Wang P, Woodruff TK, Rojans I, Byrne MC, Mat-

- zuk MM 1999 Transgenic models to study gonadotropin function: the role of follicle-stimulating hormone in gonadal growth and tumorigenesis. *Mol Endocrinol* 13:851-865.
27. Kumar TR, Low MJ, Matzuk MM 1998 Genetic rescue of follicle stimulating hormone  $\beta$  deficient mice. *Endocrinology* 139:3289-3295.
  28. Weiss J, Axelrod L, Whitcomb RW, Harris PE, Crowley WF, Jameson JL 1992 Hypogonadism caused by a single amino acid substitution in the  $\beta$  subunit of luteinizing hormone. *N Engl J Med* 326:179-183.
  29. Zhang FP, Poutanen M, Wilbertz J, Huhtaniemi I 2001 Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LHRKO) mice. *Mol Endocrinol* 15:172-183.
  30. Singh J, O'Neill C, Handelsman DJ 1995 Induction of spermatogenesis by androgens in gonadotrophin-deficient (hpg) mice. *Endocrinology* 136:3311-3321.
  31. Ebling FJ, Brooks AN, Crumlin AS, Ford H, Kerr JB 2000 Estrogenic induction of spermatogenesis in the hypogonadal mouse. *Endocrinology* 141:2861-2869.
  32. Shenker A, Laue L, Kusugi S, Merendino Jr H, Minegishi T, Culler Jr CB 1993 A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. *Nature* 365:652-654.
  33. Risma KA, Clay CM, Nett TM, Wagner T, Yun J, Nilson JH 1995 Targeted overexpression of luteinizing hormone in transgenic mice leads to infertility, polycystic ovaries, and ovarian tumors. *Proc Natl Acad Sci USA* 92:1322-1326.
  34. Matzuk MM, Hsueh AJ, Layolt P, Tsafiri A, Keene JL, Boime I 1990 The biological role of the carboxyl-terminal extension of human chorionic gonadotropin  $\beta$  subunit. *Endocrinology* 126:376-383.
  35. Boime I, Ben-Menahem D 1999 Glycoprotein hormone structure-function and analog design. *Recent Prog Horm Res* 54:271-288.
  36. Keri KA, Lozada KI, Abdul-Karim FW, Nadeau JN, Nilson JH 2000 Luteinizing hormone induction of ovarian tumors: oligogenic differences between mouse strains dictates tumor disposition. *Proc Natl Acad Sci USA* 97:384-387.
  37. Risma KA, Hirschfield AN, Nilson JH 1997 Elevated luteinizing hormone in prepubertal transgenic mice causes hyperandrogenemia, precocious puberty, and substantial ovarian pathology. *Endocrinology* 138:3540-3547.
  38. Gore-Langton RE, Armstrong DT 1994 Follicular steroidogenesis and its control. In: Knobil E, Neill J, eds. *The physiology of reproduction*. 2nd ed. New York: Raven Press, Ltd, vol 1:571-677.
  39. Hall PF 1994 Testicular steroid synthesis: organization and regulation. In: Knobil E, Neill J, eds. *The physiology of reproduction*. 2nd ed. New York: Raven Press, Ltd, vol 1:1335-1362.
  40. Lubahn DB, Moyer JS, Gulding TS, Couse JE, Korach KS, Smithies O 1993 Alteration of reproductive function but not prenatal sexual development after neonatal disruption of the mouse estrogen receptor gene. *Proc Natl Acad Sci USA* 90:11162-11166.
  41. Schumberg DW, Couse JE, Mukherjee A, Lubahn DB, Sar M, Mayo KE, Korach KS 1999 Targeted disruption of the estrogen receptor  $\alpha$  gene in female mice: characterization of ovarian responses and phenotype in the adult. *Endocrinology* 140:2733-2744.
  42. Scully KM, Gleiberman AS, Lindzey J, Lubahn DB, Korach KS, Rosenfeld MG 1997 Role of estrogen receptor- $\alpha$  in the anterior pituitary gland. *Mol Endocrinol* 11:674-681.
  43. Couse JE, Korach KS 1999 Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev* 20:358-417.
  44. Couse JE, Bunch DO, Lindzey J, Schumberg DW, Korach KS 1999 Prevention of the polycystic ovarian phenotype and characterization of ovulatory capacity in the estrogen receptor  $\alpha$  knockout mouse. *Endocrinology* 140:5855-5867.
  45. Couse JE, Curtis Hewitt S, Korach KS 2000 Receptor null mice reveal contrasting roles for estrogen receptor  $\alpha$  and  $\beta$  in reproductive tissues. *J Steroid Biochem Mol Biol* 74:287-296.
  46. Couse JE, Hewitt SC, Bunch DO, Sar M, Walker VR, Davis BJ, Korach KS 1999 Postnatal sex reversal of the ovaries in mice lacking estrogen receptors  $\alpha$  and  $\beta$ . *Science* 286:2323-2326.
  47. Hess RA, Bunick D, Lee KH, Bahr J, Taylor JA, Korach KS, Lubahn DB 1997 A role for estrogens in the male reproductive system. *Nature* 390:509-512.
  48. Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS 1994 Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med* 331:1056-1061.
  49. Ohlsson C, Hellberg N, Parini P, Vidal O, Bohlooly M, Rudling M, Lindberg MK, Warner M, Angelin B, Gustafsson JA 2000 Obesity and disturbed lipoprotein profile in estrogen receptor- $\alpha$  deficient male mice. *Biochem Biophys Res Commun* 278:640-645.
  50. Fisher CR, Graves KH, Parlow AE, Simpson ER 1998 Characterization of mice deficient in aromatase (AroKO) because of targeted disruption of the *cyp19* gene. *Proc Natl Acad Sci USA* 95:6965-6970.
  51. Britt KL, Drummond AE, Cox VA, Dyson M, Wreford NG, Jones ME, Simpson ER, Findlay JK 2000 Androgen-related ovarian phenotype in mice with targeted disruption of the *Cyp 19* (aromatase) gene. *Endocrinology* 141:2614-2623.
  52. Robertson KM, O'Donnell J, Jones ME, Meachem SJ, Boon WC, Fisher CR, Graves KH, McLachlan RI, Simpson ER 1999 Impairment of spermatogenesis in mice lacking a functional aromatase (*cyp 19*) gene. *Proc Natl Acad Sci USA* 96:7986-7991.
  53. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K 1995 Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metab* 80:3689-3698.
  54. Li X, Nakkala E, Yan W, Streng T, Saarinen N, Warri A, Huhtaniemi I, Santti R, Makela S, Poutanen M 2001 Altered structure and function of reproductive organs in transgenic male mice overexpressing human aromatase. *Endocrinology* 142:2435-2442.
  55. Bosland MC 1996 Hormonal factors in carcinogenesis of the prostate and testis in humans and in animal models. *Prog Clin Biol Res* 341:309-352.
  56. Fowler KA, Gill K, Kirma N, Dillehay DL, Tekmal RR 2000 Overexpression of aromatase leads to development of testicular Leydig cell tumors: an *in vivo* model for hormone-mediated testicular cancer. *Am J Pathol* 156:347-353.
  57. Tekmal RR, Ramachandra N, Gubba S, Durgam VR, Mantione J, Toda K, Shizuta Y, Dillehay DL 1996 Overexpression of *int-5/aromatase* in mammary glands of transgenic mice results in the induction of hyperplasia and nuclear abnormalities. *Cancer Res* 56:3180-3185.
  58. Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y 2000 Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology* 141:1317-1324.
  59. Vale W, Bilezikjian LM, Rivier C 1994 Reproductive and other roles of inhibins and activins. In: Knobil E, Neill J, eds. *The physiology of reproduction*. 2nd ed. New York: Raven Press, Ltd, vol 1:1861-1878.
  60. Matzuk MM, Finegold MJ, Su J-CJ, Hsueh AJW, Bradley A 1992  $\alpha$ -inhibin is a tumor-suppressor gene with gonadal specificity in mice. *Nature* 360:313-319.
  61. Matzuk MM, Finegold MJ, Mather JP, Krummen L, Lu H, Bradley A 1994 Development of cancer cachexia-like syndrome and adrenal tumors in  $\alpha$ -inhibin-deficient mice. *Proc Natl Acad Sci USA* 91:8817-8821.
  62. Matzuk MM, Kumar TR, Shou W, Coerver KA, Lau AL, Behringer RR, Finegold MJ 1996 Transgenic models to study the roles of inhibins and activins in reproduction, oncogenesis, and development. *Recent Prog Horm Res* 51:123-157.
  63. Kumar TR, Wang Y, Matzuk MM 1996 Gonadotropins are essential modifier factors for gonadal tumor development in inhibin deficient mice. *Endocrinology* 137:4210-4216.
  64. Cho BN, McMullen MI, Pei L, Yates CJ, Mayo KE 2001 Reproductive deficiencies in transgenic mice expressing the rat inhibin  $\alpha$  subunit gene. *Endocrinology* 142:4994-5001.
  65. Shelling AN, Burton KA, Chand AL, van de CC, France JT, Farquhar CM, Milson SR, Love DR, Gonsak K, Aittomaki K, Winship IM 2000 Inhibin: a candidate gene for premature ovarian failure. *Hum Reprod* 15:2644-2649.
  66. Kananen K, Markkula M, Rainio E, Su JG, Hsueh AJ, Huhtaniemi IT 1995 Gonadal tumorigenesis in transgenic mice bearing the mouse inhibin  $\alpha$ -subunit promoter/simian virus 40 T-antigen fusion gene: characterization of ovarian tumors and establishment of gonadotropin-responsive granulosa cell lines. *Mol Endocrinol* 9:616-627.
  67. Kananen K, Markkula M, Mikola M, Rainio EM, McNeilly A, Huhtaniemi I 1996 Gonadectomy permits adrenocortical tumorigenesis in mice transgenic for the mouse inhibin  $\alpha$ -subunit promoter/simian virus 40 T-antigen fusion gene: evidence for negative autoregulation of the inhibin  $\alpha$ -subunit gene. *Mol Endocrinol* 10:1667-1677.
  68. Rahman NA, Huhtaniemi IT 2001 Ovarian tumorigenesis in mice transgenic for murine inhibin  $\alpha$  subunit promoter-driven Simian Virus 40 T-antigen: ontogeny, functional characteristics, and endocrine effects. *Biol Reprod* 64:1172-1179.
  69. Kihlanawati, Kero J, Paukku T, Huhtaniemi I 2000 Long-term testosterone treatment prevents gonadal and adrenal tumorigenesis of mice transgenic for the mouse inhibin  $\alpha$  subunit promoter/simian virus 40 T-antigen fusion gene. *J Endocrinol* 166:77-85.
  70. Kihlanawati, Paukku T, Kero J, Zhang FP, Rahman N, Kananen K, Huhtaniemi I 1998 Direct luteinizing hormone action triggers adrenocortical tumorigenesis in castrated mice transgenic for the murine inhibin  $\alpha$ -subunit promoter/simian virus 40 T-antigen fusion gene. *Mol Endocrinol* 12:801-809.
  71. Kero J, Poutanen M, Zhang FP, Rahman N, McNeilly AM, Nilson JH, Keri KA, Huhtaniemi IT 2000 Elevated luteinizing hormone induces expression of its receptor and promotes steroidogenesis in the adrenal cortex. *J Clin Invest* 105:633-641.
  72. Brown CW, Houston-Hawkins DE, Woudruff TK, Matzuk MM 2000 Insertion of *Inhib* into the *Inhb* locus rescues the *Inhb* null phenotype and reveals new activin functions. *Nat Genet* 25:453-457.
  73. Vassalli A, Matzuk MM, Gardner HAK, Lee K-P, Jaenisch R 1994 Activin/inhibin  $\beta$  subunit gene disruption leads to defects in cyclid development and female reproduction. *Genes Dev* 8:414-427.
  74. Nishimori K, Young LJ, Guo Q, Wang Z, Insel TR, Matzuk MM 1996 Oxytocin is required for nursing but is not essential for parturition or reproductive behavior. *Proc Natl Acad Sci USA* 93:11699-11704.
  75. Vale W, Hsueh A, Rivier C, Yu J 1990 In: Sporn MB, Roberts AW, eds. *Peptide growth factors and their receptors*. Berlin: Springer-Verlag, 419-477.
  76. Matzuk MM, Kumar TR, Bradley A 1995 Distinct phenotypes for mice deficient in either activins or activin receptor type II. *Nature* 374:396-399.

2834 Endocrinology, August 2002, 143(8):2823-2835

Burns and Matsuoka • Mini-review

77. Nakamura T, Takio K, Eto Y, Shibai H, Titani K, Sugino H 1990 Activin binding protein from rat ovary is follistatin. *Science* 247:836-838
78. Matsuoka MM, Lu H, Vogel H, Seilheimer K, Rupp DR, Bradley A 1995 Multiple defects and perinatal death in mice deficient in follistatin. *Nature* 372:360-363
79. Guo Q, Kumar TR, Woodruff T, Hadsell LA, DeMayo FJ, Matsuoka MM 1998 Overexpression of mouse follistatin causes reproductive defects in transgenic mice. *Mol Endocrinol* 12:96-106
80. Luo X, Ikeda Y, Parker KL 1994 A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* 77:481-490
81. Ikeda Y, Luo X, Abbud R, Nilson JH, Parker KL 1995 The nuclear receptor steroidogenic factor-1 is essential for the formation of the ventromedial hypothalamic nucleus. *Mol Endocrinol* 9:478-486
82. Ingraham HA, Lala DS, Ikeda Y, Luo X, Shen WH, Nachtigal MW, Abbud R, Nilson JH, Parker KL 1994 The nuclear receptor steroidogenic factor 1 acts at multiple levels of the reproductive axis. *Cancer Res* 54:2302-2312
83. Zhao L, Bakke M, Parker KL 2001 Pituitary-specific knockout of steroidogenic factor 1. *Mol Cell Endocrinol* 185:27-32
84. Kendall SK, Saunders TL, Jin L, Lloyd RV, Glode LM, Nett TM, Keri RA, Nilson JH, Camper SA 1991 Targeted ablation of pituitary gonadotropins in transgenic mice. *Mol Endocrinol* 5:2029-2036
85. Achermann JC, Hu M, Hindmarsh PC, Jamieson JL 1999 A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. *Nat Genet* 22:125-126
86. Nachtigal MW, Hirokawa Y, Eynart-VanHouten DL, Hanagan JN, Hammer GD, Ingraham HA 1998 Wilms' tumor 1 and Dax-1 modulate the orphan nuclear receptor SF-1 in sex-specific gene expression. *Cell* 93:435-454
87. Pelletier J, Bruening W, Kashtan CE, Mauer SM, Manivel JC, Striegel JE, Houghton DC, Junien C, Habib R, Fouser L, Fine RN, Silverman BL, Hausman D 1991 Germ-line mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. *Cell* 67:437-447
88. Bardoni B, Zanaria L, Caioli S, Floridia G, Worley KC, Tonini G, Ferrante E, Chiunello G, McCabe BK, Fracarro M, Zuffardi O, Camerino G 1994 A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. *Nat Genet* 7:497-501
89. Palek CE, Little ML, Fleming S, Miles C, Charlier JP, Clarke AR, Miyagawa K, Christie S, Doig J, Harrison DJ, Porteous DJ, Brookes AJ, Hooper ML, Hastie ND 1999 A zinc finger truncation of murine WT1 results in the characteristic urogenital abnormalities of Denys-Drash syndrome. *Proc Natl Acad Sci USA* 96:2931-2936
90. Swain A, Narvaez V, Burgoyne P, Camerino G, Lovell-Badge R 1998 Dax1 antagonizes sex action in mammalian sex determination. *Nature* 391:761-767
91. Whitworth DJ, Behringer RR 2001 The transgenic mouse in studies of mammalian sexual differentiation. In: Matsuoka MM, Brown CW, Kumar TR, eds. *Transgenics in endocrinology*. Totowa, NJ: Humana Press; 19-39
92. Acampora D, Mazan S, Avantsaggiato V, Barone P, Tuorto F, Lallemand Y, Brulet P, Simeone A 1996 Epilepsy and brain abnormalities in mice lacking the *Uchl1* gene. *Nat Genet* 14:218-222
93. Acampora D, Mazan S, Tuorto F, Avantsaggiato V, Tremblay JJ, Lazzaro D, di Carlo A, Mariano A, Macellia PL, Corte G, Macellia V, Drouin J, Brulet P, Simeone A 1998 Transient dwarfism and hypogonadism in mice lacking *Uchl1* reveal pubertal stage-specific control of pituitary levels of GH, IGF-I and LH. *Development* 126:1229-1239
94. Lee SL, Sadovsky Y, Swinuff AH, Polish JA, Goda P, Gavrilina G, Milbrandt J 1996 Late-inacting hormone deficiency and female infertility in mice lacking the transcription factor NG2A (Egr3). *Science* 273:1719-1721
95. Topilko P, Schneider-Maunoury S, Levi G, Tremblay JJ, Gourdji D, Driancourt MA, Rao CV, Charnay P 1998 Multiple pituitary and ovarian defects in *Krox-20* (*NG2A*, *Egr3*) targeted mice. *Mol Endocrinol* 12:107-122
96. Schneider-Maunoury S, Topilko P, Seitanidou T, Levi G, Cohen-Tannoudji M, Pournin S, Babinet C, Charnay P 1993 Disruption of *Krox-20* results in alteration of rhombomeres 3 and 5 in the developing hindbrain. *Cell* 75:1199-1214
97. Topilko P, Schneider-Maunoury S, Levi G, Baron-Van Evercooren A, Chennoufi AB, Seitanidou T, Babinet C, Charnay P 1994 *Krox-20* controls myelination in the peripheral nervous system. *Nature* 371:706-709
98. Tourtellotte WC, Milbrandt J 1998 Sensory axon and muscle spindle agenesis in mice lacking the transcription factor *Egr3*. *Nat Genet* 2:87-91
99. Tourtellotte WC, Nagarajan R, Ayyang A, Mueller C, Milbrandt J 1999 Infertility associated with incomplete spermatogenic arrest and oligospermia in *Egr4* deficient mice. *Development* 126:5061-5071
100. Tourtellotte WC, Nagarajan R, Bartke A, Milbrandt J 2000 Functional compensation by *Egr4* in *Egr3*-dependent fertilizing hormone regulation and Leydig cell steroidogenesis. *Mol Cell Biol* 20:5261-5268
101. Hocheraude-Reviens MT, de Reviens MM, Monet-Kuntz C, Perreau C, Fontaine J, Viguerie-Martinez MC 1987 Testicular growth and hormonal parameters in the male Snell dwarf mouse. *Acta Endocrinol (Copenh)* 115:399-405
102. de Reviens MM, Viguerie-Martinez MC, Mariana JC 1984 FSH, LH and prolactin levels, ovarian follicular development and ovarian responsiveness to FSH in the Snell dwarf mouse. *Acta Endocrinol (Copenh)* 106:121-126
103. Tang K, Bartke A, Cardiner CS, Wagner TE, Yun JS 1993 Gonadotropin secretion, synthesis, and gene expression in human growth hormone transgenic mice and in Ames dwarf mice. *Endocrinology* 132:2518-2524
104. Parks JS, Brown MR, Hurley DJ, Phelps CJ, Wajnaraj MP 1998 Heritable disorders of pituitary development. *J Clin Endocrinol Metab* 84:4362-4371
105. Stahl H, Kendall SK, Brinkmeier ML, Greco TL, Watkins-Chow DE, Campos-Barros A, Lloyd RV, Camper SA 1999 Thyroid hormone is essential for pituitary somatotropes and lactotropes. *Endocrinology* 140:1884-1892
106. Krassas GE 2000 Thyroid disease and female reproduction. *Fertil Steril* 74:1067-1070
107. Krassas GE, Pontikides N, Kaltsas T, Papadopoulos P, Paunovic J, Paunovic N, Duntas LH 1999 Disturbances of menstruation in hypothyroidism. *Clin Endocrinol (Oxf)* 50:655-659
108. Krassas GE, Pontikides N, Kaltsas T, Papadopoulos P, Patrinos M 1994 Menstrual disturbances in thyrotoxicosis. *Clin Endocrinol (Oxf)* 40:641-644
109. Jiang JY, Inai Y, Umezaki M, Sato E 2001 Characteristics of infertility in female hypothyroid (hvt) mice. *Reproduction* 122:695-700
110. Milenkovic L, D'Angelo G, Kelly PA, Weiner RI 1994 Inhibition of gonadotropin hormone-releasing hormone release by prolactin from CTT neuronal cell lines through prolactin receptors. *Proc Natl Acad Sci USA* 91:1244-1247
111. Horsman ND, Zhao W, Monterino-Rodriguez E, Tanaka M, Nakashima K, Single SJ, Smith T, Markoff E, Donahue K 1997 Defective mammatogenesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. *EMBO J* 16:6926-6935
112. Ormandy CJ, Camus A, Barra J, Damotte D, Lucas B, Bouteau H, Ederly M, Brousse N, Babinet C, Binaut N, Kelly PA 1997 Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. *Genes Dev* 11:167-178
113. Danilovich N, Wernsing D, Coschicagno KT, Kopechick JJ, Bartke A 1999 Defects in female reproductive function in C11-OR-KO mice; role of KGF-1. *Endocrinology* 140:2637-2640
114. Chandrasekar V, Bartke A, Awoniyi CA, Tsai-Morris CII, Dufau ML, Russell LD, Kopechick JJ 2001 Testicular endocrine function in C11 receptor gene disrupted mice. *Endocrinology* 142:3443-3450
115. Creim M, Fadden C, Keen J, Steger RW, Bartke A 1995 Infertility in transgenic mice overexpressing the bovine growth hormone gene: disruption of the neuroendocrine control of prolactin secretion during pregnancy. *Biol Reprod* 52:1187-1192
116. Danilovich NA, Bartke A, Winters TA 2000 Ovarian follicle apoptosis in bovine growth hormone transgenic mice. *Biol Reprod* 62:103-107
117. Eisenhauer KM, Chun SY, Billig H, Hsueh AJ 1995 Growth hormone suppression of apoptosis in preovulatory rat follicles and partial neutralization by insulin-like growth factor binding protein. *Biol Reprod* 53:13-20
118. Katanasich L, Kleinberg D, Vance ML, Stravou S, Palaski KJ, Schoenfeld DA, Hayden DL, Wright ME, Woodburn CJ, Klibanski A 2001 Hypogonadism in patients with acromegaly: data from the multi-center acromegaly registry pilot study. *Clin Endocrinol (Oxf)* 54:183-188
119. Baker J, Hardy MF, Zhou J, Bondy C, Lupu F, Bellvé AR, Lifshatz A 1996 Effects of an *Igf1* gene null mutation on mouse reproduction. *Mol Endocrinol* 10:903-918
120. Zhou J, Kumar TR, Matsuoka MM, Bondy C 1997 Insulin-like growth factor I regulates gonadotropin responsiveness in the murine ovary. *Mol Endocrinol* 11:1924-1933
121. Klinger B, Anin S, Silbergeld A, Eshet R, Laron Z 1998 Development of hyperandrogenism during treatment with insulin-like growth factor-I (IGF-I) in female patients with Laron syndrome. *Clin Endocrinol (Oxf)* 48:81-87
122. Laron Z, Klinger B 1998 Effect of insulin like growth factor-I treatment on serum androgens and testicular and penile size in males with Laron syndrome (primary growth hormone resistance). *Eur J Endocrinol* 138:176-180
123. Kumar TR, Wiseman AL, Kala G, Kala SV, Matsuoka MM, Lieberman MW 2000 Reproductive defects in  $\gamma$ -glutamyl transpeptidase-deficient mice. *Endocrinology* 141:4270-4277
124. Swerdloff RS, Peterson M, Vera A, Batt RA, Heber D, Bray GA 1978 The hypothalamic-pituitary axis in genetically obese (ob/ob) mice: response to luteinizing hormone releasing hormone. *Endocrinology* 103:542-547
125. Swerdloff RS, Batt RA, Bray GA 1976 Reproductive hormonal function in the genetically obese (ob/ob) mouse. *Endocrinology* 98:1359-1364
126. Chua Jr SC, Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, Leibel RL 1996 Phenotypes of mouse diabetes and fat mice due to mutations in the *Ob* (leptin) receptor. *Science* 271:994-996
127. Yu WH, Kimura M, Walewska A, Karanth S, McCann SM 1997 Role of leptin in hypothalamic-pituitary function. *Proc Natl Acad Sci USA* 94:1023-1028
128. Tena-Sempere M, Manna PR, Zhang FP, Pinilla L, Gonzalez LC, Dieguez C, Huhtaniemi I, Aguilar E 2001 Molecular mechanisms of leptin action in adult rat testis: potential targets for leptin-induced inhibition of steroidogenesis and pattern of leptin receptor messenger ribonucleic acid expression. *J Endocrinol* 170:413-423
129. Greisen S, Ledet T, Moller N, Jorgensen JO, Christiansen JS, Petersen K,



- Ovesen P 2000 Effects of leptin on basal and LH-stimulated steroidogenesis in human granulosa luteal cells. *Acta Obstet Gynecol Scand* 79:931–935
- 130 Kruse M, Bornstein SR, Uhlmann K, Paeth G, Scherbaum WA 1998 Leptin down-regulates the steroid producing system in the adrenal. *Endocr Res* 23:887–900
- 131 Spicer LJ, Francis CC 1998 Adipose obese gene product, leptin, inhibits bovine ovarian thecal cell steroidogenesis. *Biol Reprod* 58:207–212
- 132 Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF 1996 Serum immunoreactive leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295
- 133 Valentino R, Savastano S, Tommaselli AP, D'Amore G, Dorato M, Lombardi G 2001 The influence of intense ballet training on trabecular bone mass, hormone status, and gonadotropin structure in young women. *J Clin Endocrinol Metab* 86:4674–4678
- 134 Tommaselli AP, Valentino R, Savastano S, Randazzo G, Scalfi L, Contaldo F, Dorato M, Lombardi G 1995 Altered glycosylation of pituitary gonadotropins in anorexia nervosa: an alternative explanation for amenorrhea. *Eur J Endocrinol* 132:450–455
- 135 Farooqi IS, Keogh JM, Kamath S, Jones S, Gibson WT, Trussell R, Jebb SA, Lip GY, O'Rahilly S 2001 Partial leptin deficiency and human adiposity. *Nature* 414:34–35
- 136 Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S 1997 Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 387:905–908
- 137 Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gommelen M, Dina C, Chambaz J, Lacorte JM, Bandevant A, Bougneres P, Lebour Y, Froguel P, Guy-Girard B 1998 A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 392:398–401
- 138 Chanson P, De Roux N, Young J, Bidart JM, Jaquet P, Miralhi M, Milgrom E, Schaison G 1998 Absence of activating mutations in the GPR11 receptor gene in human pituitary gonadotroph adenomas. *Eur J Endocrinol* 139: 152–160
- 139 Matthews CH, Borgato S, Beck-Peccoz P, Adams M, Tunc Y, Gambino G, Casagrande S, Tedeschi G, Benedetti A, Chatterjee VK 1993 Primary amenorrhea and infertility due to a mutation in the  $\beta$  subunit of follicle-stimulating hormone. *Nat Genet* 5:83–86
- 140 Layman LC, Lee EJ, Peak DB, Naminoum AB, Vu KV, van Lingen BL, Gray MR, McDonough PG, Reindollar KH, Jameson JL 1997 Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone  $\beta$  subunit gene. *N Engl J Med* 337:607–611
- 141 Aittomaki K, Lucena JLD, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J, Kaskikari R, Sankila E-M, Nieschlag E, Hultaniemi L, Chapelle AD 1995 Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypogonadotropic ovarian failure. *Cell* 82:979–988
- 142 Gromoll J, Simoni M, Nieschlag E 1996 An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. *J Clin Endocrinol Metab* 81:1367–1370
- 143 Latronico AC, Anastasi A, Arnold R, Rapaport R, Mendonca BB, Bloise W, Castro M, Tsigos C, Chrousos CP 1996 Brief report: testicular and ovarian resistance to luteinizing hormone caused by inactivating mutations of the luteinizing hormone receptor gene. *N Engl J Med* 334:507–512
- 144 Kremer H, Kraaij R, Toledo SP, Post M, Fridman JB, Hayashida CY, van Reem M, Milgrom E, Rogers III, Mariman E, Themmen APN, Brunner HG 1997 Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nat Genet* 9:160–164
- 145 Toledo SP, Brunner HG, Kraaij R, Post M, Dahia PL, Hayashida CY, Kremer H 1996 An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46, XX female. *J Clin Endocrinol Metab* 81:3850–3854
- 146 Tanimoto Y, Tanimoto K, Sugiyama E, Horiguchi H, Murakami K, Yagami K, Fukamizu A 1999 Male sterility in transgenic mice expressing activin  $\beta$ A subunit gene in testis. *Biochem Biophys Res Commun* 259:699–705
- 147 Bartke A, Ceeim M, Tang K, Steger KW, Chandrasekar V, Turyn D 1994 Neuroendocrine and reproductive consequences of overexpression of growth hormone in transgenic mice. *Proc Soc Exp Biol Med* 206:345–350
- 148 Strauss BL, Pittman R, Pixley MR, Nilson JH, Boime I 1994 Expression of the  $\beta$  subunit of chorionic gonadotropin in transgenic mice. *J Biol Chem* 269:4968–4973
- 149 Yura S, Ogawa Y, Sagawa N, Masuzaki H, Itoh H, Ebihara K, Aizawa-Abe M, Fujii S, Nakan K 2000 Accelerated puberty and late-onset hypothalamic hypogonadism in female transgenic skinny mice overexpressing leptin. *J Clin Invest* 105:749–755
- 150 Pierson MP, DeMayo FJ, Maltzuk MM, Tsai SY, O'Malley BW 2000 Regulated expression of inhibin A in wild-type and inhibin  $\alpha$  null mice. *Mol Endocrinol* 14:1075–1085
- 151 Radovick S, Wray S, Lee F, Nicols DK, Nakayama Y, Weintraub BD, Westphal H, CuDer Jr GB, Wondisford FE 1991 Migratory arrest of gonadotropin-releasing hormone neurons in transgenic mice. *Proc Natl Acad Sci USA* 88:3402–3406
- 152 Kananen K, Markkula M, el-Hefnawy T, Zhang FP, Paukku T, Su JG, Hsueh AJ, Huttaniemi L 1996 The mouse inhibin  $\alpha$ -subunit promoter directs SV40 T antigen in Leydig cells in transgenic mice. *Mol Cell Endocrinol* 119: 135–146
- 153 Yu RN, Ito M, Saunders TL, Camper SA, Jameson J 1998 Role of Alch in gonadal development and gametogenesis. *Nat Genet* 20:353–357
- 154 Kregel JH, Hodge JB, Couse JE, Enmark E, Warner M, Mahler JE, Sari M, Korach KS, Gustafsson J-A, Smithes O 1998 Generation and reproductive phenotypes of mice lacking estrogen receptor  $\beta$ . *Proc Natl Acad Sci USA* 95:15677–15682
- 155 Zhou Y, Xu BC, Maheshwari HC, He F, Reed M, Lozykowski M, Okada S, Cataldo L, Cuschigano K, Wagner TE, Baumann G, Kopchick JJ 1997 A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). *Proc Natl Acad Sci USA* 94:13215–13220
- 156 St John MA, Tao W, Fei X, Fukumoto R, Carcangiu ML, Brownstein DK, Parlow AF, McGrath J, Xu T 1999 Mice deficient of Lats1 develop soft-tissue sarcomas, ovarian tumours and pituitary dysfunction. *Nat Genet* 21:182–186
- 157 Lei ZM, Mishra S, Zou W, Xu B, Foltz M, Li X, Rao CV 2001 Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. *Mol Endocrinol* 15:189–200
- 158 Gaud DJ, Porter ED, Mahon KA, Parlow AF, Westphal H, Kirsch IR 1997 Hypogonadism and obesity in mice with a targeted deletion of the Nhlh2 gene. *Nat Genet* 15:397–401
- 159 Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Kruse W, Muller-Wieland D, Kahn CR 2000 Role of brain insulin receptor in control of body weight and reproduction. *Science* 289:2122–2125
- 160 Li S, Crenshaw 3rd EB, Rawson EJ, Simmons DM, Swanson LW, Rosenfeld MG 1990 Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene pit-1. *Nature* 347:528–533
- 161 Sonson MW, Wu W, Daxen JS, Flynn SE, Norman DJ, O'Connell SM, Gukovsky I, Cardiere C, Ryan AK, Miller AP, Zuo L, Gleiberman AS, Andersen B, Beamer WC, Rosenfeld MG 1996 Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. *Nature* 384:322–333
- 162 Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman DJ, Jaenisch R 1993 WT-1 is required for early kidney development. *Cell* 74: 679–691



## review

## Genetic dissection of mammalian fertility pathways

Martin M. Matzuk\*†‡§ and Dolores J. Lamb†§

Departments of \*Pathology, †Molecular and Cellular Biology and ‡Molecular and Human Genetics, and §Scott Department of Urology, Baylor College of Medicine, Houston, TX 77030, USA

E-mail: mmatzuk@bcm.tmc.edu

The world's population is increasing at an alarming rate and is projected to reach nine billion by 2050. Despite this, 15% of couples world-wide remain childless because of infertility. Few genetic causes of infertility have been identified in humans; nevertheless, genetic aetiologies are thought to underlie many cases of idiopathic infertility. Mouse models with reproductive defects as a major phenotype are being rapidly created and discovered and now total over 200. These models are helping to define mechanisms of reproductive function, as well as identify potential new contraceptive targets and genes involved in the pathophysiology of reproductive disorders. With this new information, men and women will continue to be confronted with difficult decisions on whether or not to use state-of-the-art technology and hormonal treatments to propagate their germline, despite the risks of transmitting mutant genes to their offspring.

Despite advances in assisted reproductive technologies, infertility is a major health problem worldwide. Approximately 15% of couples are unable to conceive within one year of unprotected intercourse. The fertility potential of a couple is dependent on the coordinated and combined functions of both male and female reproductive systems. Anatomic defects, gametogenesis dysfunction, endocrinopathies, immunologic problems, ejaculatory failure and environmental exposures are significant causes of infertility. Although several infertility disorders are associated with defined genetic syndromes (for example, cystic fibrosis and Turner's Syndrome<sup>1,2</sup>), almost a quarter of clinical infertility cases of either sex are idiopathic in nature, in part as a result of a poor understanding of the basic mechanisms regulating fertility. It is thought that genetic defects underlie many of these unrecognized pathologies. On the basis of over 200 infertile or subfertile genetic mouse models (see Supplementary Information Table; also see ref. 3), it is not surprising that the diagnosis of idiopathic infertility is common in the clinic<sup>4,5</sup>.

In this review, we discuss causes of mammalian infertility with an emphasis on the genetic basis of fertility defects in humans and mice. Animal models have defined key signalling pathways and proteins involved in reproductive physiology<sup>6</sup>. Mouse models

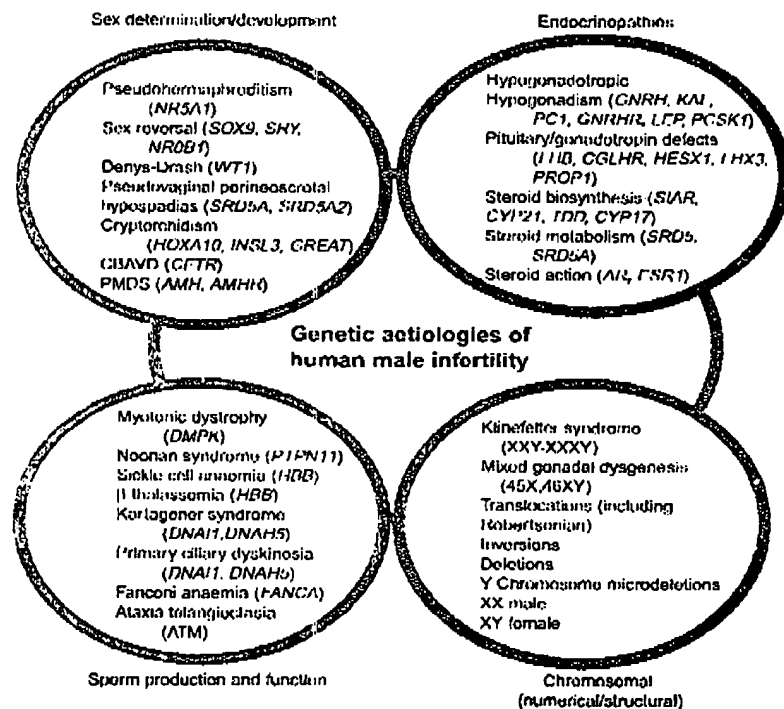
have been produced by spontaneous mutations, fortuitous transgene integration, retroviral infection of embryonic stem cells, ethylnitrosourea (ENU) mutagenesis and gene targeting technologies<sup>7,8</sup>. These mutations affect all aspects of reproduction, including ovarian development and function, testis determination, spermatogenesis, sperm function, genital tract development and function, sexual behaviour, fertilization and early embryonic development, and therefore have contributed much to our understanding of infertility. For example, male infertility is observed in the spontaneous mutant models hypogonadal (*hpg*)<sup>9</sup> and testicular feminization (*tfn*)<sup>10</sup> and in models created by transgene integration, such as the *kisimo* mouse model (which arose by transgene disruption of the *Thy1* gene<sup>11</sup>) and retroviral disruption of the *Behr*<sup>12</sup>, *Mtap*<sup>13</sup> and *Spm*<sup>14</sup> genes. These models are improving our knowledge of the genetic basis of mammalian infertility and suggest that in the future, clinical technologies must advance to enable analysis of many more genes when an infertile couple enters the clinic. Currently, karyotype analysis, sequence analysis of the cystic fibrosis transmembrane conductance regulator gene and Y chromosome deletion analysis (for males) are the only genetic tests commonly offered to infertile patients<sup>4,5</sup>.

## Where it all begins

Reproductive development and physiology are evolutionarily conserved processes across eutherian mammalian species and many other vertebrates, including marsupials<sup>15</sup>, amphibians, reptiles, birds and fish<sup>16–19</sup>. Several genes required for vertebrate fertility are also highly conserved in evolution, with orthologues in *Drosophila melanogaster* (for example, *vasa* (DDX4), *fat facets* (DDFRY) and *boule* (DAZ)<sup>20–22</sup>). Propagation of the vertebrate germline requires development of the gonads, the site of future gamete production. The indifferent gonad forms during foetal development, primordial germ cells enter the gonad primordium and the tissue eventually differentiates along a female (ovarian) or male (testicular) pathway; this differentiation dictates the formation of the secondary sex organs<sup>23</sup>. Although there may be spatiotemporal variations of these processes in different species (for example, in mice and humans, gonadal sex determination occurs *in utero*, whereas in marsupial mammals, it occurs after birth), they eventually yield ovaries that produce eggs or testes that generate spermatozoa.

Defects in sexual differentiation pathways can cause infertility in mice and humans of both sexes (Fig. 1)<sup>24–26</sup>. In 1959, through the analysis of human XO (Turner syndrome) females and XXY

## fertility supplement



**Figure 1 Genetic aetiologies of human male infertility.** Developmental disorders causing human male infertility result from a failure of gonadal development or testis determination, endocrinopathies, well known genetic syndromes, and numerical and structural chromosomal abnormalities (translocations, deletions and inversions).

(Klinefelter syndrome) males, as well as XO and XX female mice and XY male mice, it was concluded that the Y chromosome was male determining<sup>26–28</sup>. Subsequent chromosomal and genetic studies of humans and mice with sex reversal syndromes and infertility revealed that many XX males have translocations of a small piece of the Y chromosome, so that the sex determining region Y gene (*SRY*) results in testis development. Similarly, XY females often have inactivating mutations in the *SRY* gene, resulting in the development of ovaries<sup>29,30</sup>. A critical role of *Sry* in sex determination was confirmed by showing that the expression of an *Sry* transgene in an XX mouse causes testis formation, and physical and behavioural sex reversal<sup>31</sup>. Most *SRY* mutations disrupt the high mobility group (HMG) box of

the *SRY* protein; not surprisingly, this region is highly conserved among different species<sup>32</sup>. HMG box-containing proteins typically bind and significantly bend DNA and function as transcription factors or facilitators of transcription. Several genes upstream and downstream of *SRY* in the sex determination pathway are now known (reviewed in ref. 23). For example, XY female sex reversal correlates with a duplication of the human X-linked gene *DAX1* (ref. 33) or haploinsufficiency of the autosomal *SOX9* gene<sup>32,34–36</sup>. Interestingly, whereas an extra Y chromosome (that is, XYY) has little effect on human male fertility because of the selected loss of the extra Y during spermatogenesis<sup>37</sup>, Klinefelter (XXY XXXY) males account for 10–15% of azoospermic patients<sup>38</sup>.

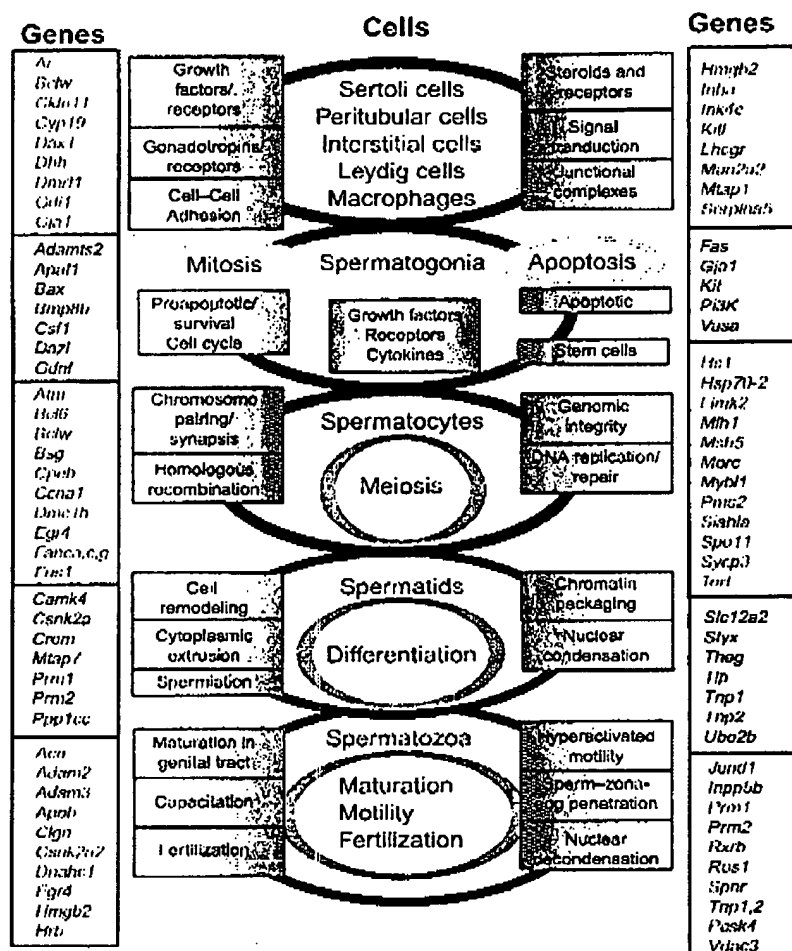
## From a distance, they will come

In both sexes, the primordial germ cells (PGCs) are defined histologically as alkaline-phosphatase-positive embryonic cells<sup>39,40</sup>. In the mouse, these cells divide rapidly under the influence of transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily signals; knockout models lacking bone morphogenetic protein-4 (BMP-4) or BMP-8b, or the downstream cytoplasmic-to-nuclear relay proteins, SMAD1 and SMAD5, have defects in PGC development<sup>41–44</sup>. At mid-gestation, the PGCs begin one of the longest journeys of any mammalian cell, migrating from their origin at the base of the yolk sac, along the hind-gut, to eventually enter the genital ridge. Factors required for this migration in humans are unknown, although chemoattractants and cell adhesion factors have been implicated<sup>45</sup>. In the mouse, mutations in Kit receptor (*KITR*) and Kit ligand (*KITL*) genes block PGC migration, causing infertility, but not altering sexual differentiation<sup>46</sup>.

Few known human mutations result in a reduction of the PGC or follicle pool, although girls with Turner's syndrome (partial or complete X-chromosome monosomy), have streak (remnant) gonads with no oocytes. Many Turner's syndrome cases with ovarian failure seem to be caused by loss of the short arm of the X chromosome<sup>4</sup>. Among the candidate Turner's syndrome ovarian failure genes are *ZFX*, *BMP15*, *UBE1* and *USP9X* (ref. 2). An absence of *Zfx* in mice results in a loss of germ cells and subfertility in both sexes<sup>47</sup>; loss of *BMP15* in sheep causes a block at the primary follicle stage and infertility<sup>48</sup>. Studies in XO mice suggest that abnormal chromosomal segregation contributes to germ cell problems<sup>49</sup>, indicating that multiple factors are responsible for these human ovarian abnormalities.

## Death in the germline

In females, quiescent primordial follicles (a non-growing oocyte surrounded by squamous granulosa cells) form during prenatal life in humans and post-natally in mice. Recruitment of these follicles during



**Figure 2 Genes Involved in the regulation of male reproduction in the mouse.** Spermatogenesis requires a complex interaction of the various cellular compartments of the testis (seminiferous epithelium containing spermatogenic cells, Sertoli cells and peritubular myoid cells, the interstitial cell compartment containing the steroidogenic Leydig cells, macrophages, and other interstitial cells, and the vasculature). Targeted mutation of the genes shown affects specific testicular cell types and reproductive function, resulting in male infertility or subfertility in the mouse (detailed in the Supplementary Information Table).

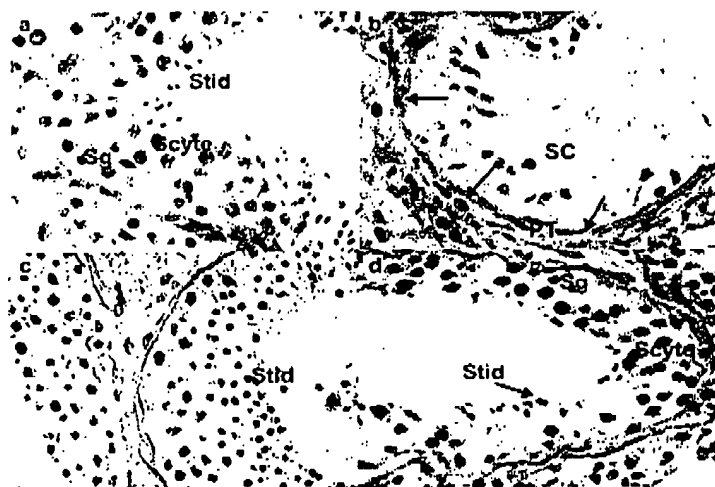
ovarian folliculogenesis permits further growth and development of oocytes. In males, spermatogenesis is characterized by three specific functional phases: proliferation, meiosis and spermiogenesis. The proliferative phase in the testis begins early in embryonic development, and with the exception of a brief period when spermatogonia arrest during late foetal and early postnatal life, they proliferate actively

throughout life. Spermatogonial stem cells were one of the first recognized examples of adult stem cells capable of rejuvenating spermatogenesis after toxic insult<sup>24,25</sup>. In contrast, the formation of primordial follicles in females defines a finite endowment of oocytes. Between the time of ovary development and reproductive sequence, there is a precipitous drop in the number of oocytes. In humans, seven million foetal

germ cells at 20 weeks are reduced to two million oocytes at birth, and eventually to 300,000 at puberty<sup>2,3,4</sup>. Thus, factors that prenatally and postnatally regulate germ cell survival in the ovary can prolong the reproductive lifespan.

The spermatogonia proliferation rate, the highest in the body, is well regulated; thus, it is not surprising that genes involved in growth (for example, *Ki*, *Csf* and *Bmp8b*) and apoptosis are also required for normal spermatogonia (Fig. 2). This stage of spermatogenesis is also noteworthy for its inefficiency; in rats, 75% of spermatogonia do not survive to become mature sperm<sup>24</sup>. A balance of anti-apoptotic members of the BCL2 family (that is, BCL2, BCL6, BCLX, and BCLW) and the pro-apoptotic BAX protein is extremely important in the regulation of germ cell survival prenatally and postnatally in both sexes, and in response to toxins in the ovary<sup>25,26</sup>. It is possible that defects in this delicate balance of cell proliferation and cell death contribute to the clinical pathology of hypospermatogenesis (all cellular elements of the testis are present, but at low cellularity). In the mouse, an absence of BCLX results in a complete loss of germ cells before birth in both males and females; furthermore, a lack of BCLW results in a partial reduction of PGCs in females, whereas an absence of BCL2 results in only decreased oocyte numbers postnatally<sup>12,26,27</sup>. Absence of either BAX or BCLW causes male infertility, and absence of BCL6 causes male subfertility, again suggesting that a balance of apoptotic/anti apoptotic factors is necessary for normal spermatogenesis. In contrast, a prolonged female reproductive lifespan occurs in the absence of BAX in mice, consistent with its pro-apoptotic role<sup>28</sup>. Polycyclic aromatic hydrocarbons in cigarette smoke and air pollution bind to the aryl hydrocarbon receptor to stimulate transcriptional activation of *Bax*, thereby enhancing apoptosis and oocyte loss<sup>24</sup>. In the future, factors that inhibit the BAX pathways or stimulate the anti-apoptotic pathways could prolong the reproductive lifespans of women.

## fertility supplement



**Figure 3 Spermatogenic failure in the human.** Spermatogenesis in the human is characterized by six stages that are present in a mosaic fashion in the seminiferous tubule. **a**, Normal human spermatogenesis with Sertoli cells (SC) spermatogonia (Sg) towards the basal portion of the tubule, spermatocytes (Scyto) and maturing spermatic (Stid) located towards the lumen of the tubule, the tubules are surrounded by peritubular myoid cells (PT). The interstitial area contains the steroidogenic Leydig cells that secrete testosterone. **b**, An example of the most severe testicular pathology, with a total absence of germ cells and a Sertoli cell only pathology. Mild thickening of the peritubular layer is also observed (arrows: peritubular fibrosis). **c**, A late maturation arrest. The most mature cell type present is the round spermatid. **d**, An example of hypospERMatogenesis, where all cell types are present in the testis, but with a low level of cellularity within the seminiferous tubule.

### Meiosis, recombination, the integrity of the genome and more death

Meiosis is a process of cell division that is unique to germ cells and is required for the production of healthy haploid gametes (reviewed in greater detail in ref. 62). This process is evolutionarily important for both the integrity and diversity of species, as recombination of homologous chromosomes occurs during prophase of the first meiotic division, helping to orient chromosomes on the meiotic spindle, as well as introducing genetic variability. Male spermatogenesis is initiated postnatally (in mice at postnatal day 7) and is a continuous process producing spermatozoa. Proliferating spermatogonia differentiate and enter meiosis as spermatocytes. In contrast, oogenesis is initiated prenatally (in mice at embryonic day 13), arrests initially at the diplotene stage of meiotic prophase, resumes with the preovulatory luteinizing

hormone (LH) surge and arrests again after the first polar body is released before fertilization.

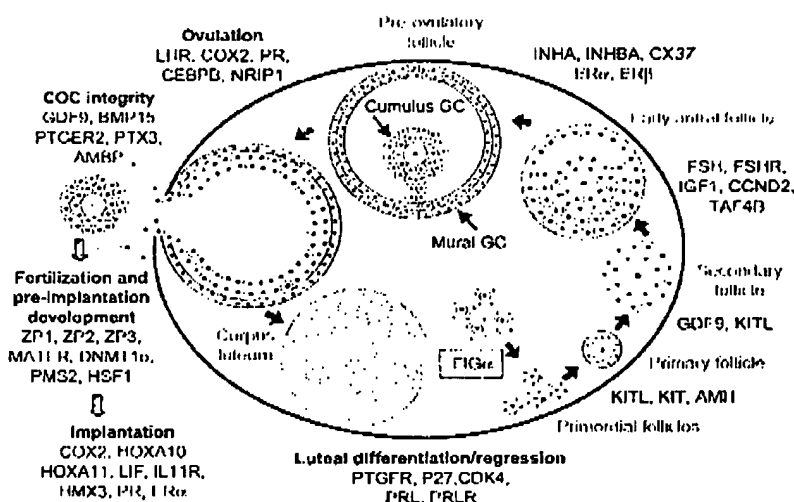
Despite sexual dimorphism in meiosis, many regulators of the process are common to the germ cells of both sexes. In the absence of these proteins, prophase arrest and accompanying germ cell death occur in male and female germ cells. Infertility in both sexes is observed in knockout mice lacking the recombination and DNA damage/mismatch repair proteins, SPO11, DMCI, ataxia telangiectasia (ATM), MSH4, MLI1, and MSH5 (refs 63–74). Mutations in *ATM* and Fanconi anaemia (*FA*) complementation-group-protein genes result in fertility defects in humans and mice of both sexes (Figs 1,2). *ATM* is involved in DNA metabolism and cell cycle checkpoint control<sup>65</sup>, whereas *FA* is a hereditary chromosomal instability syndrome<sup>66</sup>. *FA* men are hypogonadal, oligospermic and rarely fertile; *FA* women

can experience premature ovarian failure in their 20s. Several *FA* mouse models have been created and display reduced fertility<sup>77</sup>. Thus, similar mechanisms for germline monitoring are conserved in mammals and in both sexes.

When the germline 'proofreading' system goes awry in an otherwise 'normal' individual, there are major consequences. Despite a normal somatic karyotype, sperm collected from oligospermic men exhibit an increased frequency of chromosomal abnormalities<sup>78,79</sup>. Aneuploidy is the most common genetic abnormality in humans<sup>80</sup>, and the common trisomies (for example, trisomy 21 (Down's syndrome and trisomy 18)) arise primarily in the children of ageing women through non-disjunction defects during the first meiotic division<sup>81</sup>. These findings are exemplified in mice lacking synaptonemal complex protein 3 (*SYCP3*), which functions in synapsis (pairing) of the homologous chromosomes during meiosis. *Sycp3* knockout males are infertile; females are subfertile, exhibiting loss of aneuploid embryos<sup>82,83</sup>. Interestingly, germline deletions resulting in Duchenne muscular dystrophy (*DM1*) more often arise during oogenesis, whereas *DM1* point mutations result more commonly from spermatogenic failure<sup>84</sup>. This suggests that some proofreading mechanisms during male and female gametogenesis may differ (see also ref. 80).

### Hormones take control

After sexual maturity, all stages of spermatogenesis (male) and folliculogenesis (female) are observed, the end result in each case being gamete production. Hypothalamic-pituitary control of gonadal somatic cells is critical for fertility in all mammals and in both sexes (Fig. 3; reviewed in refs 85–88). Gonadotropin releasing hormone (*GnRH*) from the hypothalamus regulates the pituitary gonadotrope production of follicle stimulating hormone (*FSH*) and LH,  $\alpha$ / $\beta$  heterodimers that share a common  $\alpha$  subunit with placental human chorionic gonadotropin (*hCG*) and pituitary thyroid stimulating hormone (*TSH*). Spontaneous deletion of the hypothalamic *GnRH* encoding sequences in mice (that is, *hpg*),



**Figure 4 Female fertility proteins.** Knockout mouse models have defined key proteins that function at various stages of follicle formation, folliculogenesis, ovulation, and post-ovulatory events. *FIGA* is required for primordial follicle formation, and several proteins are needed for oocyte and granulosa cell (GC) growth and differentiation, ovulation, and the integrity of the cumulus oocyte complex (COC) (reviewed in the Supplementary information table).

mutation of the human GnRH processing enzyme gene (*PC1*), disruption of developmental migration of the GnRH neurons in human Kallman Syndrome, or mutation of the GnRH receptor gene (expressed in gonadotropes), results in hypogonadotropic hypogonadism (HHG) and infertility (Fig. 1). Loss-of-function mutations in the pituitary expressed *FSH1B* genes and gonadal-expressed *FSH1* receptor genes decrease testis size and spermatozoa counts in men and male mice, and cause a block in folliculogenesis and infertility in women and female mice. This emphasizes the conservation and importance of these signalling pathways. Similarly, pituitary gland development and downstream steroidogenic pathways are conserved in humans and mice and are critical for fertility in both sexes. For example, a homozygous *Propt* missense mutation causes multiple pituitary defects in the *Ames dwarf* mouse, including defects in gonadotrope differentiation and infertility in all female and most male mice. Similarly, human *PROT1* mutations cause combined pituitary hormone deficiency, including HHG and infertility (Fig. 1).

Members of the steroid receptor super

family and their transcriptional coactivators (for example, AR, ER, PR, RXR $\beta$ , SF1, DAX1, and SRC1) are pivotal in the regulation of reproductive function. Disruption of any of the genes involved in androgen biosynthesis, metabolism and action negatively impact male development, spermatogenesis and function. Spontaneous mutations of the X-linked androgen receptor gene in XY mice (that is, *tfm* (testicular feminization)) and humans result in individuals with abnormal testes, no ductal system and external female genitalia<sup>14,99</sup> (<http://www2.mcgill.ca/androgendb>). Absence of steroid 5 $\alpha$  reductase, which converts testosterone to dihydrotestosterone, results in external female genitalia, prostate absence in XY humans and developmental disruption of the male ductal system (that is, seminal vesicles and prostate)<sup>90,91</sup>. Similarly, mutations in the orphan nuclear receptors, steroidogenic factor-1 (SF1) and DAX1, have been described in mice and humans; mutations in *DAX1* cause almost universal HHG in adult humans (Fig. 1).

Not surprisingly, oestrogen and progesterone are key to early folliculogenesis and

corpus luteum maintenance of early pregnancy in the female<sup>92</sup>. Targeted deletion of oestrogen receptor  $\alpha$  in mice revealed that it is also required for male fertility and for male and female sexual behaviour<sup>93</sup>. Similarly, the progesterone receptor (also required for female fertility) is important in sexual behaviour in the mouse<sup>94</sup>. In the evaluation of the infertile couple, assessment of circulating hormone levels (FSH, LH, testosterone, prolactin and free testosterone in the male; FSH, LH, oestradiol and progesterone levels in the female) can provide important information concerning the function of the hypothalamic–pituitary–gonadal axis and the presence of endocrinopathies.

## Spermatogenesis has many unique players

Spermatogenesis requires not only the appropriate hormonal milieu, but also autocrine, paracrine and juxtacrine signalling between the various testicular compartments. The testis is composed of an interstitial cell compartment with androgen producing Leydig cells, and the seminiferous tubule containing Sertoli cells, peritubular myoid cells and germ cells. Whereas follicles are recruited each cycle to enter the ovulatory pathway in females, all stages of spermatogenesis are present at any one time in different tubules within the testis. Thus, the wave of spermatogenesis resulting in development of mature sperm is a spatial cycle rather than a temporal one. The importance of growth factors and cytokines, their receptors and signal transduction pathways to gametogenesis cannot be underestimated. For example, deletion of mouse *Desert hedgehog* (*Dhh*) affects testicular development, resulting in anastomatic seminiferous tubules and an absence of adult Leydig cells. Similarly, the insulin-like growth factor (*Igf1*) null male mouse is characterized by vestigial vas deferens, prostate and seminal vesicles, caused by a steroidogenic Leydig cell defect.

Once male germ cells complete meiosis to achieve a haploid chromosomal complement, they are called spermatids. Spermatids undergo a process of cellular differentiation known as spermiogenesis, progressing from round to elongating to

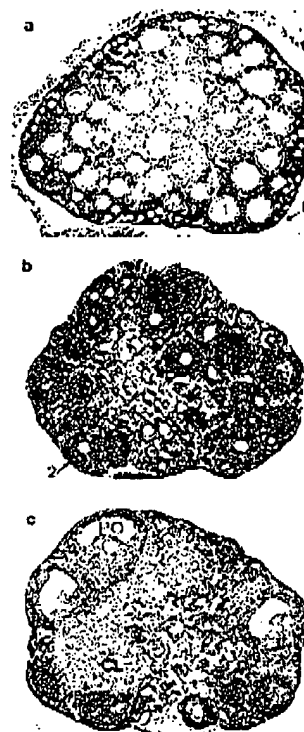
## fertility supplement

elongated spermatids, culminating in the development of spermatozoa. Many male-specific genes are involved in this extensive cellular remodelling and concomitant condensation of the chromatin (for example, *Tnp1*, *Tnp2*, *Ptn1*, *Ptn2*, *Thg* and *Hsp60-2*; see Fig. 2). In common with many of the Y chromosome genes that encode RNA-binding proteins and are implicated in human infertility, an absence of similar proteins in the mouse, such as STYX, disrupts spermatid development<sup>90</sup>. After spermiogenesis and release of the spermatozoa from the Sertoli cells into the seminiferous tubule lumen, acquisition of motility occurs during transit through the epididymis and capacitation occurs in the oviduct (fallopian tubes) of the female genital tract. Both of these processes are required for effective penetration of the zona pellucida and egg.

In the evaluation of the infertile male, a semen sample is ordered to determine sperm count, motility and morphology. Some laboratories perform sperm function tests that predict defects in sperm-zona or egg interaction, or in penetration. However, semen analysis is not a definitive test of the fertility potential of an individual unless there are no sperm in the ejaculate. This is also true in mice. For example, *FSH-β* mutant mice exhibit reduced sperm counts, but fertility is normal<sup>91</sup>. Conversely, many mouse models are infertile and demonstrate abnormal sperm function, sperm motility (for example, *ApoB*, *CatSper*, *Dnahc1*, *Hmgb2* and *Ros* knockout mice), or morphology (for example, *Tnp2*, *Tnp1* and *Sperm1* knockout mice) with no detrimental effect on sperm count. In addition, targeted deletion of the *Ace*, *Adam2*, *Adam3*, *calnegin*, *Pe4*, *Span1*, *Spmr*, *Thg26*, *Id2* or *Mllc7* genes results in normal sperm count, motility and morphology; however, sperm function is defective (Fig. 2). As a majority of unexplained cases of infertility in human males result from spermatogenic defects (Fig. 3), the homologues of the above-described mouse genes are actively being pursued for their possible roles in human infertility.

Chromosomal abnormalities are observed in 5.8% of infertile males<sup>92</sup> and

**Figure 5 Mouse knockout models to study folliculogenesis.** **a**, Targeted mutation of the oocyte-secreted growth factor, *Gdf9*, results in an early folliculogenesis block, resulting in an ovary with only primordial (P) and primary (1) follicles<sup>100</sup>. **b**, Absence of the endocrine hormone, FSH, results in a later block at the secondary (2) to antral follicle transition<sup>98</sup>. **c**, These knockout models contrast with wild-type ovaries that contain preovulatory (PO) follicles and corpora lutea (CL). Primary to preovulatory-stage oocytes are surrounded by a zona pellucida (magenta).



more commonly involve sex chromosomes (4.2%), as opposed to autosomes (1.5%; Fig. 1). In addition to *SRY*, other Y chromosome genes are required for spermatogenesis. This became obvious in the XX *Sry* male mouse and the XX *Sry* transgene positive male mouse, which are sex reversed, but display spermatogenesis blocks<sup>93</sup>. Similarly, a region at Yq11 that is deleted in several infertile men was termed the azoospermia factor (*AZF*) region<sup>94</sup>. In general, the reported incidence of deletions in this region in severe oligospermic/azoospermic men is 10–18% and varies depending on the stringency of diagnostic classification<sup>95</sup>. This region (now further subdivided into *AZF<sub>a</sub>*, *AZF<sub>b</sub>* and *AZF<sub>c</sub>*), contains several genes involved in spermatogenesis, including deleted in azoospermia (*DAZ*)<sup>106,107</sup>. In the mouse, disruption of the testis-expressed *Dazl* homologue gene on chromosome 17 abrogates gamete production. Other putative evolutionarily conserved spermatogenesis genes have been mapped to Y chromosome regions commonly deleted in infertile men (reviewed in ref. 99). Mutations in the human gene *USP9Y* (ubiquitin specific protease 9, Y chromosome or DIFY), a homologue of the *D. melanogaster* development *fat facets* gene<sup>21</sup>, cause infertility<sup>108</sup>. Functional analysis of additional Y chromosome genes in the mouse has been complicated by the presence of multiple copies or X-chromosome homologues, as well as technical difficulties related to the low efficiency of Y chromosome homologous recombination in embryonic stem cells. However, it is expected that the recent and exciting technological breakthroughs achieved by Bishop and colleagues<sup>109</sup>, who developed a

method for successful gene targeting of the Y chromosome in embryonic stem cells, and the development of an embryonic stem cell line<sup>109</sup> that will facilitate germ line transmission of Y chromosome targeted genes, will rapidly translate into an enhanced understanding of the role of specific Y chromosome genes in male reproductive function.

### No crosstalk in females, no folliculogenesis progression

Although several proteins are involved in ovarian folliculogenesis, meiosis, and oocyte survival, oocyte-somatic cell crosstalk is especially critical for release of a fertilizable egg (Fig. 4 and ref. 105). Without the helix-loop-helix protein factor in the germline  $\alpha$  (*FIG $\alpha$* ), pre-granulosa (somatic) cells fail to form a monolayer around individual primordial oocytes, resulting in rapid germ cell depletion from the neonatal mouse ovary and sexual infantilism<sup>106</sup>. Similarly, oocyte growth during folliculogenesis is regulated by signalling of

granulosa KITL to the oocyte expressed KITR<sup>tm</sup>. KITL expression is controlled by both hormonal (FSH) and oocyte (growth differentiation factor 9 (GDF-9)) factors<sup>102</sup>. In the absence of the TGF- $\beta$  superfamily ligand GDF-9 in mice<sup>103</sup> or its close oocyte-specific homologue, BMP-15, in sheep<sup>98</sup>, an arrest in folliculogenesis at the primary follicle stage is observed (Fig. 5). FSH has no effect on the 'arrested' primary follicles of *Gdf9* knockout ovaries<sup>104</sup>, suggesting that GDF-9 allows the granulosa cells to grow and acquire the competence to respond to FSH. Absence of GDF-9 results in elevated levels of KITL<sup>tm</sup>, which signals back to markedly increase oocyte size<sup>106</sup>. These findings were confirmed by studies showing that recombinant GDF-9 downregulates levels of *Kitl* mRNA<sup>107</sup>. In addition to oocyte factors, FSH<sup>tm</sup> functions with IGF-1 (by stimulating cyclin D2 and oestrogen synthesis)<sup>108,111</sup> to regulate the growth of the follicle through the pre-ovulatory stage (Fig. 5). In pre-ovulatory follicles, LH, in conjunction with the oocyte-secreted proteins GDF-9 and BMP-15, signals to somatic cells to initiate ovulation of a healthy cumulus oocyte complex (COOC). Thus, important crosstalk between somatic cells and oocytes, as well as endocrine signalling, is necessary for normal folliculogenesis and ovulation.

Post-fertilization, *Mater* (maternal antigen that embryos require) and several other genes (including *Dnmt1a*, *Pms2* and *Hsf1*) (Fig. 4), have been identified by knockout mouse studies as maternal effect (oocyte synthesized) genes that are essential for development<sup>112</sup>. The human homologue of *Mater* has been identified and may be a candidate gene for premature ovarian failure<sup>113</sup>. Similarly, several uterine proteins are required for implantation (Fig. 1 and ref. 114). Thus, these studies have pinpointed multiple putative diagnostic targets in women who present with infertility.

In women, several syndromes — including ovarian failure and infertility — are attributed to autosomal recessive mutations<sup>96,115</sup>. Blepharophimosis/ptosis/epicanthus inversus syndrome, the only autosomal dominant disorder associated with premature ovarian failure (POF), is caused

by mutations in the forkhead transcription factor gene (*FOXO2*)<sup>116</sup>. Expansion of a CCG trinucleotide repeat of the Xq27.3 fragile X mental retardation gene (*FMR1*) to over 200 repeats is the most common heritable cause of mental retardation. The unstable premutation *FMR1* allele (60–199 CCG repeats) causes POF in 21% of heterozygote carriers and increased twin pregnancies<sup>117</sup>. Furthermore, 2% of sporadic cases and 14% of familial cases of POF are associated with the premutation allele. The pathophysiology of the premutation allele in POF is unknown, but this finding clearly represents a step forward in identifying a genetic locus for POF. To date, all other identified single gene autosomal dominant or recessive mutations with isolated infertility in humans affect steroidogenic or gonadotropin pathways, often in both sexes. However, many candidate genes await analysis in human idiopathic infertility cases.

### Descent of the testis and problems with sperm transit

Testis determination and gametogenesis are necessary, but not sufficient, for male fertility, as testicular descent down the inguinal canal into the scrotum, in addition to the development of the genital tract and penis, are also critical. Mutations of the mouse genes *Ins3*, *Great* (G protein coupled receptor that affects testicular descent; a possible relaxin receptor) and *Hoxa10* (refs 118–122) result in male infertility secondary to cryptorchidism. The second phase of testicular descent requires androgens and a functional androgen receptor. In humans, cryptorchidism results from anti-Müllerian hormone (AMH) deficiency caused by obstruction of the genital tract.

Gonadal sex determines secondary duct differentiation. In females, the Müllerian duct differentiates into the oviducts, uterus and upper portion of the vagina; in males, the Wolffian duct differentiates into epididymis, vas deferens and seminal vesicles<sup>19,23,123</sup>. The Müllerian duct regresses in response to prenatal production of testicular AMH, and Wolffian duct development requires testosterone. Differentiation of the prostate and male external genitalia is

driven by dihydrotestosterone, a product of the conversion of testosterone by 5  $\alpha$  reductase. Mutations in genes that affect steroidogenesis (for example, P450 aromatase (*CYP19*) and 5- $\alpha$  reductase) and steroid signalling pathways (for example, oestrogen receptor  $\alpha$  (*ER $\alpha$* ) and androgen receptor) have deleterious effects on genital tract development and function in the male. Thus, it follows that pseudohermaphroditism occurs as a result of defects in genes involved in gonad formation (for example, *SP1* and *WT1*). Mutations of the AMH or AMH receptor genes result in persistence of Müllerian duct syndrome (PMDS), resulting in obstructive azoospermia and fertility defects in men, male dogs and mice (Fig. 1 and ref. 123).

One to two per cent of infertile men present with obstructive azoospermia caused by congenital bilateral absence of the vas deferens (CBAVD), as a result of mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene<sup>124</sup>. These CBAVD patients successfully father off spring because microsurgical epididymal sperm aspiration yields 'normal' sperm for *in vitro* fertilization (IVF). Male fertility also may be compromised by epididymal, ejaculatory or erectile dysfunction, as well as by other congenital anomalies.

### New technologies and perspectives

Genome, gene and cDNA sequences are being deposited into public databases (for example, the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov>) or the Wellcome Trust Sanger Institute (<http://www.sanger.ac.uk>)) with amazing speed. Furthermore, programs to search these databases, such as BLAST (<http://www.ncbi.nlm.nih.gov/blast>) and the Unigene database at NCBI, are helping scientists to use this wealth of information. In particular, sequences unique to mammalian germ cells have been identified using an *in silico* subtraction (electronic database) approach<sup>125</sup>. For example, *GASZ* (germ-cell-specific and ankyrin repeat, sterile  $\alpha$  motif and basic leucine-zipper-containing protein) was identified as a

## fertility supplement

novel evolutionarily-conserved germ cell-expressed gene lying adjacent to the *CRT* gene in human, chimpanzee, baboon, cow, rat and mouse<sup>18</sup>. Functional expression and sequence data is also being collated into collections, such as the Ovarian Kaleidoscope database (<http://ovary.stanford.edu>), the Male Reproductive Genetics database (<http://mrp.genetics.washington.edu/home.html>) and the GermOnline database (<http://germonline.igh.cnrs.fr>). With the use of microarrays for expression analysis of reproductive tissues<sup>12</sup>, these 'bits' of data will increase exponentially. Therefore, there is an urgent need for bioinformatics advances to facilitate compiling and sorting through this wealth of *in silico* information for future applications in the clinic.

Technological procedures and advances in the clinic are also wrought with some controversies and dilemmas. Of particular importance, the application of assisted reproductive technologies (ART) for severe male and female factor infertility serves to not only overcome sterility, but bypasses natural barriers to the inheritance of defective genes. This results in considerable concern that genetic defects will be transmitted to the next generation. Among these controversial treatments are ICSI (intracytoplasmic sperm injection), cytoplasmic (ooplasmic) transfer, round spermatid nuclear injection (ROSI or ROSI) and reproductive cloning, (described in more detail by Schatten in this issue<sup>19</sup>).

These technologies have prompted significant debates concerning their morality and safety. However, even a United States government moratorium on human cloning has not deterred renegade scientists overseas from actively engaging in this research, which could result in a potentially disastrous outcome.

## Conclusions

To date, diagnosis of infertility in the clinic has been hindered by our relatively poor understanding of the underlying molecular mechanisms. Although investigators have attempted to translate findings in animal models to humans by searching for gene mutations/deletions in idiopathic infertility

patients, in general, these investigations have not been fruitful. Given the large number of candidate evolutionarily conserved 'fertility' genes yet to be discovered or identified from mutant mouse studies, and the overall complexity of the reproductive system in general, proper diagnosis and treatment of these patients will await the development of more sophisticated and rapid technologies. Finally, if mutation of a gene in mice or humans results in infertility, the protein product of that gene may be a future target for novel contraceptives that are designed to transiently or permanently cause infertility. J

1. Amikam, A. *et al.* Congenital bilateral absence of the vas deferens: A primarily genital form of cystic fibrosis. *J. Am. Med. Assoc.* 269, 1294–1297 (1993).
2. Zima, A. D. & Rao, J. T. Molecular analysis of genes on Xp22.3 controlling fertility syndrome and premature ovarian failure (POF). *Hum. Reprod.* 14, 141–146 (1999).
3. Buxis, K., DeLacy, L. J. & Matzuk, M. M. *Reproductive Medicine: Molecular, Cellular and Genetic Fundamentals* (ed. Fauser, B. C. F. M.) (Ch. 10) (Springer Publishing, Box 17, Basel, CH, 2001).
4. Epshel, I. & Hirsch, G. *Infertility in the male* (Harley Press, St. Louis, MO, 1997).
5. Crespiani, P. C. & Rutim, R. T. Optimal use of infertility diagnostic tests and resources: The EMRI Capri Workshop Group. *Hum. Reprod.* 15, 223–232 (2000).
6. *Infertility in Endocrinology* (Ed. Hirsch, G.) (Tübingen, CH, 2001).
7. Halling, R. L. NGI mutagenesis analyzing gene function in situ. *Annu. Rev. Genomics Hum. Genet.* 2, 463–491 (2001).
8. Caporaso, M. E. Consistent mice with targeted mutations. *Nature Med.* 7, 1026–1027 (2001).
9. Mason, A. *et al.* A complex human DNA sequence of human telomeres that includes short regions of satellite and homology with telomeric growth factor. *J. Nucl. Med.* 39, 66–67 (1998).
10. Chazotte, N. *et al.* A human telomere destabilization/segment recombination (TERRA) in the human genome. *Mol. Endocrinol.* 5, 573–581 (1991).
11. Yanaka, M. *et al.* Inactivation of the mouse *klf4* locus is caused by a deletion in spermatogenesis. *J. Biol. Chem.* 275, 11,791–11,794 (2000).
12. Bays, V. *et al.* Testicular degeneration in *klf4* deficient mice. *Nature Genet.* 10, 74–76 (1998).
13. Komada, M., Akizumi, T. I., Yoshida, M. M., Russell, L. D. & Sorokos, B. E. MAP-1A, encoding a microtubule-associated protein, is a testis- and oviduct-specific gene required for spermatogenesis. *Genes Dev.* 13, 1332–1341 (1999).
14. Pines, O. *et al.* A *klf4* deficient for spermatid germ line DNA binding protein shows neurological, spermatogenic, and sperm morphological abnormalities. *Dev. Biol.* 233, 319–334 (2001).
15. Bostrom, M. & Shaw, G. Germ cells, growth and sex reversal in man. *Reprod. Biol. Fert.* 26, 107–117 (2001).
16. Durr, A. *et al.* *klf4* underlies the germ line development in testes. *Am. J. Physiol.* 280, 200–204 (2001).
17. Gaffney, J. L. & Loh, P. Germ line development in vertebrates and invertebrates. *J. Cell Mol. Life Sci.* 55, 1141–1151 (1999).
18. Matzuk, M. M. & Lehman, R. Showing how it's the next generation. *Mol. Reprod.* 10, 1–10 (2001).
19. Gilbert, S. F. *Developmental Biology* (Sinauer Associates, Inc., Sunderland, 1993).
20. Yanaka, M. *et al.* The mouse homologue of *Drosophila Yawa* is required for the development of male germ cells. *Genes Dev.* 14, 811–821 (2000).
21. Jones, M. H. *et al.* The *Penelope* developmental gene, *pat* factor, for a human homologue in Xp11.1 which escapes X-inactivation and has related sequences on Yp11.1. *Hum. Mol. Genet.* 5, 1001–1004 (1996).
22. Hoshino, A. *et al.* Mann, J. Z. & Macintosh, S. A. Mouse cell cycle requirement for a fly homologue of human *Yawa* in *Drosophila*. *Nature* 381, 783–785 (1998).
23. Whitworth, C. E. & Benington, R. P. *Contemporary Endocrinology: Endocrine in Pathophysiology* (ed. Matzuk, M. M., Brown, C. W., Kumar, L. R.) (Humana Press, Totowa, NJ, 2001).
24. Madhus, M. & Lamb, D. Understanding the new genes for male infertility. *J. End.* (in press).
25. Yao, H. H., Gilman, C., Zhou, G. Q. & Le, L. The battle of the sex-repressing pathways in sex determination. *Nature Rev. Genet.* 3, 187–198 (2002).
26. Jacobs, P. A. & Stump, L. A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature* 183, 301–303 (1959).
27. Bond, C. E., Jones, K. W., Klatav, E. J., de Almeida, J. C. & Briggs, J. H. A sex chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet* 707, 711–715 (1959).
28. Weinman, W. J. & Russell, L. B. The Y chromosome as the bearer of male-determining factors in the mouse. *Proc. Natl. Acad. Sci. USA* 45, 568–569 (1959).
29. Sinclair, A. H. *et al.* A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature Med.* 3, 241 (1997).
30. Gubbay, J. *et al.* A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature* 338, 111–114 (1990).
31. Koopman, P., Gubbay, J., Vargiu, P., Camilleri, P. S. & de Rooij, R. Male development of chromosomally female mice transgenic for *Sry*. *Nature* 351, 111–113 (1991).
32. Caennaro, J. & Sinclair, A. H. Mutations in *Sry* and *SRY* testis-determining genes. *Hum. Mol. Genet.* 9, 389–393 (1999).
33. Zanatta, L. *et al.* A mouse model of the nuclear hormone receptor superfamily responsible for X-linked recessive hypogonadism. *Nature* 372, 635–641 (1995).
34. Foster, J. W. *et al.* Campomelic dysplasia and autosomal sex reversal caused by mutations in an *SRY*-related gene. *Nature* 372, 523–526 (1995).
35. Wagner, T. *et al.* Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the *SRY*-related gene *SOX9*. *Cell* 70, 1111–1120 (1992).
36. Sudhof, K., Schmitt, M. F., Hornsch, P. A. & Scherer, G. Sex reversal by loss of the terminal transactivation domain of human *SOX9*. *Nature Genet.* 13, 249–252 (1996).
37. Shi, Q. & Martin, R. H. Multicolor fluorescence *in situ* hybridization analysis of human chromosome segregation in a 12,XY male and a review of the literature. *Am. J. Med. Genet.* 93, 10–16 (2000).
38. De Brachet, M. & Day, N. C. Cytogenetic studies in male infertility: a review. *Hum. Reprod.* 6, 243–250 (1991).
39. Chiquena, A. D. The identification, origin and migration of the postnatal germ cells in the mouse embryo. *Ann. Rev. Embryol. Morphogen.* 11, 53–78 (1992).
40. Gindoff, M., Snow, M. H. & Klatav, E. J. Postnatal germ cells in the mouse embryo during gastrulation. *Development* 110, 521–528 (1990).
41. Chang, H. & Matzuk, M. M. *klf4* is required for mouse primordial germ cell development. *Mol. Cell.* 10, 101–111 (2002).
42. Erismann, K. *et al.* *klf4* is required for the generation of primordial germ cells in the mouse embryo. *Genes Dev.* 15, 433–436 (2001).
43. Herndon, K. D., Dunn, N. R. & Robertson, J. J. Mouse embryonic *klf4* Small T signals displacement in extra-embryonic tissues and germ cell formation. *Development* 128, 5699–5701 (2001).
44. Ying, Y., Liu, X. M., Matthe, A., Lawless, J. A. & Day, L. C. Expression of *klf4* for the generation of primordial germ cells in the mouse. *Mol. Endocrinol.* 14, 1075–1083 (2000).
45. White, S. Germ cells. *Can. J. Genet. Cytol.* 10, 101–115 (1960).
46. Duvic, P. & de la Haye, M. E. *Endocrine in Pathophysiology* (ed. Matzuk, M. M., Brown, C. W., Kumar, L. R.) (Humana Press, Totowa, NJ, 2001).
47. Jones, S. W. *et al.* *klf4* mutation results in small animal size and reduced germ cell number in male and female mice. *Development* 124, 2275–2281 (1997).
48. Gubbay, J. M. *et al.* Mutations in an ovic-derived growth factor gene (*HMP1*) cause increased ovulation rate and infertility in a developmentally sensitive mouse. *Nature Genet.* 25, 279–283 (2000).



- #### Acknowledgements

The authors thank S. Baker for her expert assistance in manuscript formatting, and R. Böttlinger, K. Brame, and M.R. Marfatian for critical reviews of the manuscript. We also thank K. Brame and W. Yan for help with the supplementary information Table. Studies in the Matsuk and Lamb laboratories on feeding pathways have been supported by the National Institutes of Health (grants HL03498, A. Amato, HL03067, and HL06792) and the Specialized Cooperative Units Program in Reproductive Research (grants HL02490).

Supplementary information is available at the pages at [www.nature.com/typh](http://www.nature.com/typh). All gene names are spelled out in full in the Supplementary Information Table.

Online Table: Mouse mutations causing reproductive defects. Only single mutant defects are described. Fertility defects of unknown gene origin are not described. M, male; F, female; Hetero, heterozygote phenotype

Mutant gene	Sex Affected	Reproductive Phenotype	Fertility Status	References
Aerosin ( <i>Acr</i> )	Males	Sperm are capable of binding and penetrating the zona pellucida	Delayed fertility	1
Activin receptor-type IIA ( <i>Acr2</i> )	Both	Antral follicle block in females; small testes, delayed fertility in males	Infertility (F) Subfertility (M)	2
Activin/inhibin $\beta$ 3 subunit ( <i>Inhbb</i> )	Female	Delivery and nursing defects	Subfertility	3
Acyl-CoA synthetase 4 ( <i>ACS4</i> ; <i>Fa14</i> )	Female (Hetero)	Enlarged uteri with prostaglandin accumulation	Subfertility	4
<i>Adamts1</i> (a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 1)	Female	Cystic formations in uteri; defects in preovulatory follicle development	Subfertility	5
<i>Adamts2</i> (procollagen N-proteinase)	Male	Defects in spermatogenesis; marked decrease in sperm within testes tubules	Infertility	6
ADP-ribosylation factor-like 4 ( <i>Arl4</i> )	Male	Significantly reduced testicular weights and sperm counts	Normal fertility	7
Alpha 1 microglobulin/bikunin ( <i>Amhp</i> ; Urinary trypsin inhibitor)	Female	Defects in ovulation and cumulus-oocytes complex (COC) integrity	Subfertility	8
Angiotensin-converting enzyme ( <i>Ace</i> )	Male	Compromised ability of sperm to fertilize ova	Subfertility	9
Androgen receptor ( <i>Ar</i> ; <i>tfm</i> or Testicular feminization)	Male	Feminized external genitalia; hypogonadal; cryptorchidism with a block in spermatogenesis	Infertility	10
Anti-Müllerian hormone ( <i>Amh</i> )	Both	Uteri development in males causes obstruction and secondary infertility; females exhibit early depletion of primordial follicles	Secondary Infertility	11,12
AMH receptor ( <i>Amhr2</i> )	Male	Uteri development in males causes obstruction and secondary infertility	Secondary Infertility	13
<i>Apaf1</i> (Apoptotic protease activating factor 1)	Male	Spermatogonial degeneration	Variable lethality; Infertility	14
Apolipoprotein B ( <i>Apob</i> )	Male (Hetero)	Decreased sperm count, motility, survival time, and ability to fertilize ova	Infertility	15
Aryl-hydrocarbon receptor ( <i>Ahr</i> )	Female	Early development of primordial follicles; decreased numbers of antral follicles	Subfertility	16,17
Ataxia telangiectasia ( <i>Atm</i> )	Both	Germ cells degenerate; disruptions evident in meiosis I	Infertility	18,19
ATP-binding cassette transporter 1 ( <i>Abca1</i> )	Female	Placental malformations leading to impaired embryo growth, embryo loss and neonatal death	Subfertility	20

Basigin ( <i>Bsg</i> )	Both	Defects in fertilization and implantation (F); block in spermatogenesis at metaphase I (M)	Partial lethality; Infertility	21,22
<i>Bax</i> (Bcl2-associated X protein)	Both	Premiotic arrest of spermatogenesis; increased oocytes and primordial follicles postnatally	Infertility (M)	23,24
<i>Bcl2</i> (B-cell leukemia/lymphoma 2)	Female	Fewer oocytes/primordial follicles in the post-natal ovary	Subfertility	25
<i>Bcl6</i>	Male	Apoptosis in metaphase I spermatocytes	Subfertility	26
<i>BclX</i> ( <i>Bcl2l</i> ) hypomorph	Both	PGCs are lost by E15.5	Infertility	27
<i>Bclw</i> ( <i>Bcl2l2</i> , <i>Bcl2-like 2</i> )	Both	Late meiotic arrest with loss of germ cells (M) and reduced PGC survival (F)	Infertility (M) Subfertility (F)	28
Bone morphogenetic protein 4 ( <i>Bmp4</i> )	Both	Absent primordial germ cell (PGC) population; defect in PGC development	Lethal	29
<i>Bmp8a</i>	Male	Degeneration of germ cells and epididymis	Progressive Infertility	30
<i>Bmp8b</i>	Both	Reduced or absent PGCs (developmental defect); Postnatal male germ cell proliferation/differentiation defect and spermatocyte apoptosis	Subfertility/ Infertility	31
<i>Bmp15</i>	Female	Defects in cumulus-oocyte complex (COC) formation and ovulation	Subfertility	32
BMP receptor, type IB ( <i>Bmpr1b</i> )	Female	Defects in estrous cyclicity, cumulus expansion, and endometrial gland development	Subfertility	33
Calmeglin ( <i>Clgn</i> )	Male	Defect in sperm-zona pellucida binding	Infertility	34
<i>Camk4</i> (calcium/calmodulin-dependent protein kinase IV)	Male	Impaired chromatin packaging during spermiogenesis	Infertility	35
cAMP-responsive element modulator ( <i>Crem</i> )	Male	Defective spermiogenesis with aberrant post-meiotic gene expression	Infertility	36,37
cAMP-specific phosphodiesterase type 4 ( <i>Pde4d</i> )	Female	Diminished sensitivity of the granulosa cells to gonadotropins	Subfertility	38
Casein kinase II $\alpha$ 1 ( <i>Csnk2a2</i> )	Male	Globozoospermia (no acrosomal cap)	Infertility	39
Caspase-2 ( <i>Casp2</i> )	Female	Decreased apoptosis of female germ cells	Increased Fertility	40
CatSper (putative sperm cation channel)	Male	Defects in motility and fertilization	Infertility	41
CD9 antigen ( <i>Cd9</i> )	Female	Sperm-egg binding defect	Subfertility	42
Cell division cycle 25 homolog B ( <i>Cdc25b</i> ) ( <i>Cdc25b</i> phosphatase)	Female	Oocytes are arrested in meiotic prophase, with defects in maturation promoting factor activity	Infertility	43
Centromere protein B ( <i>Cenpb</i> )	Both	Males are hypogonadal and have low sperm counts; females have strain-dependent uterine epithelium defects	Subfertility (F)	44,45

C/EPBB (CCAAT/enhancer-binding protein $\beta$ )	Female	Reduced ovulation and block in CL differentiation	Infertility	46
Claudin 11 ( <i>Cldn11</i> ; <i>Osp11</i> )	Male	No tight junctions between Sertoli cells	Infertility	47
Colony stimulating factor (macrophage) ( <i>Csf1</i> )	Both	Males have reduced testosterone; females have implantation and lactation defects	Subfertility	48
Colony stimulating factor (granulocyte-macrophage) ( <i>Csf2</i> )	Both	Mean litter size decrease with disproportionate loss of males pups (F); maternal effects most pronounced in intercrosses with knockout males	Intercrossing Subfertility	49
Connexin 37 ( <i>Gja4</i> ; <i>Cx37</i> )	Female	Defects in late folliculogenesis and oocyte meiosis	Infertility	50
Connexin 43 ( <i>Gja1</i> ; <i>Cx43</i> )	Both	Small ovaries and testes; decreased numbers of germ cells from E11.5	Neonatal lethality	51
<i>Cpeb</i> (cytoplasmic polyadenylation element binding protein)	Both	Disrupted germ cell differentiation and meiosis I synaptonemal complex formation	Infertility	52
Cut-like 1 ( <i>Cull1</i> ; <i>CIDP/Cux</i> ) truncation mutant	Male	Severely reduced male fertility	Subfertility	53
Cyclin A1 ( <i>CenA1</i> )	Male	Block in spermatogenesis before the first meiotic division	Infertility	54
Cyclin D2 ( <i>Cend2</i> )	Both	Failure of granulosa cell proliferation (F); males fertile with decreased testis size	Infertility (F)	55
Cyclin dependent kinase 4 ( <i>Cdk4</i> )	Female	Defects in the hypothalamic-pituitary-gonadal axis	Infertility	56
Cyclooxygenase 2 ( <i>Ptgs2</i> )	Female	Defects in ovulation and implantation	Most Infertile	57,58
<i>Cyp11a</i> (Cytochrome P450, 11a, cholesterol side chain cleavage)	Both	Males feminized with female external genitalia, underdeveloped sex organs; gonads degenerate	Lethal	59
<i>Cyp19</i> (Cytochrome P450, 19, aromatase)	Both	Early spermatogonial arrest, Leydig cell hyperplasia, and defects in sexual behavior (M); folliculogenesis block and ovulation defects (F)	Progressive Infertility (M); Infertility (F)	60-62
<i>Cyp40</i> (P450 25-hydroxyvitamin D-1 $\alpha$ -hydroxylase)	Female	Uterine hypoplasia and absence of CL	Infertility	63
Cyritestin ( <i>Adum3</i> )	Male	Altered sperm protein expression and adhesion defects during fertilization	Infertility	64,65
Dax1 ( <i>Nr0h1</i> )	Male	Progressive degeneration of the germinal epithelium	Infertility	66
<i>Dazl</i> (Deleted in azoospermia-like)	Both	Reduced germ cells; differentiation failure and degeneration of germ cells	Infertility	67
Desert hedgehog ( <i>Dhh</i> )	Male	Complete absence of mature sperm; defects in Sertoli-to-Leydig cell	Infertility	68,69

		signaling		
<i>Dmchl</i> (Disrupted meiotic cDNA 1 homolog)	Both	Defects in chromosome synapsis in meiosis; female germ cells degenerate during embryogenesis	Infertility	70,71
<i>Dnmt1o</i> (DNA methyltransferase)	Female	Embryos of knockout females die during gestation due to imprinting defects; maternal effect gene	Subfertility	72
DNA polymerase $\lambda$	Male	Immotile spermatozoa	Lethality; Infertility	73
Doublesex and mab-3 related transcription factor 1 ( <i>Dmrt1</i> )	Male	Defects in post-natal testes differentiation; disorganized seminiferous tubules and absence of germ cells	Infertility	74
Dynein heavy chain 7 ( <i>Dnahe1</i> )	Male	Defects in sperm flagellar motility	Infertility	75
Early growth response 1 ( <i>Egr1</i> ; NGFI-A) targeted <i>lacZ</i> insertion	Both	Lack of LH (M); downregulation of LH-R, not remedied with gonadotropin treatment (F)	Infertility	76
Early growth response 1 ( <i>Egr1</i> ) targeted <i>neo</i> insertion	Female	LH insufficiency; loss of estrous cyclicity, no CL; rescued by treatment with gonadotropins	Infertility	77
Early growth response 4 ( <i>Egr4</i> )	Male	Germ cells undergo apoptosis during pachytene stage	Infertility	78
ELKL motif kinase ( <i>Emk</i> )	Both	$\beta$ -gal gene trap insertion creates a null allele; homozygotes intercrossed are not fertile	Intercrossing Infertility	79
Empty spiracles homolog 2 ( <i>Emx2</i> )	Both	Defective development of gonads and urogenital tracts	Lethal	80
Estrogen receptor $\alpha$ ( <i>ER<math>\alpha</math></i> )( <i>Esr1</i> )	Both	Females have hemorrhagic ovarian cysts and uterine defects, decreased lordosis response; males develop disruptions of the seminiferous epithelium due to abnormal epididymal function, no ejaculations	Infertility	81-84
Estrogen receptor $\beta$ ( <i>ER<math>\beta</math></i> )( <i>Esr2</i> )	Both	Females are subfertile; males are fertile, but develop prostate hyperplasia	Subfertility	85
Fanconi anemia complementation group A ( <i>Fanca</i> )	Both	Hypogonadism, reduced fertility, more dramatic and progressive in females	Subfertility	86
Fanconi anemia complementation group C ( <i>Fancc</i> )	Both	Hypogonadism, compromised gametogenesis	Subfertility	87,88
Fanconi anemia complementation group G ( <i>Fancg</i> )	Both	Hypogonadism, compromised gametogenesis	Subfertility	89
Fertilin $\beta$ ( <i>Adam2</i> )	Male	Altered sperm protein expression and adhesion defects during fertilization	Infertility	65,90
Fibroblast growth factor 9 ( <i>Fgf9</i> )	Male	XY male-to-female sex reversal; phenotype ranges from testicular hypoplasia to complete sex reversal	Lethal	91
<i>Figla</i> or <i>FIGA</i> (Factor	Female	No primordial follicles develop at birth	Infertility	92

in the germline $\alpha$ )				
Fragile-X mental retardation syndrome 1 homolog ( <i>Fmr1</i> )	Male	and oocytes die Macroorchidism		93
FSH hormone $\beta$ -subunit ( <i>Fshb</i> )	Both	Female pre-antral block in folliculogenesis; males decreased testis size	Infertility (F)	94
FSH receptor ( <i>Fshr</i> )	Both	Female pre-antral block in folliculogenesis; males decreased testis size	Infertility (F)	95
<i>Fus1</i> (translocated in liposarcoma; TLS)	Male	Defects in spermatocyte chromosome pairing	Infertility	96
$\beta$ 1,4-Galactosyltransferase	Both	Male infertility; defects in sperm-egg interaction; females exhibit defects in delivery and lactation	Variable lethality	97,98
$\gamma$ -Glutamyl transpeptidase ( <i>Ggt</i> )	Both	Both males and females are hypogonadal and infertile; phenotype corrected by feeding mice N-acetylcysteine	Infertility	99,100
<i>Gdi1</i> (guanosine diphosphate dissociation inhibitor 1; Rho GDI $\alpha$ )	Both	Impaired spermatogenesis, vacuolar degeneration in males; post-implantation pregnancy defects in females	Infertility	101
Glial cell line-derived neurotrophic factor ( <i>Gdnf</i> )	Male (Hetero)	Depletion of stem cell reserves; spermatogonia differentiate	Fertile	102
Glycoprotein hormone $\alpha$ -subunit ( <i>Cga</i> )	Both	Hypogonadal due to FSH and LH deficiency	Infertility	103
<i>Gpr106</i> (G protein-coupled receptor 106)	Male	<i>Cxsp</i> males homozygous for transgene integration exhibit a high intraabdominal position of the testes, complete sterility	Infertility	104
Growth differentiation factor-7 ( <i>Gdf7</i> )	Male	Defects in seminal vesicle development	Infertility	105
Growth differentiation factor-9 ( <i>Gdf9</i> )	Female	Folliculogenesis arrest at the one-layer follicle stage	Infertility	106,107
Growth hormone receptor ( <i>Ghr</i> )	Female	Delayed puberty and prolonged pregnancy		108
<i>H19</i>	Female (Hetero)	Loss of maternal allele in developing embryos causes somatic overgrowth due to loss of IGF2 imprinting		109
Heat shock protein 70-2 ( <i>Hsp70-2</i> )	Male	Meiosis defects and germ cell apoptosis	Infertility	110
Heatshock transcription factor 1 ( <i>Hsf1</i> )	Female	Maternal effect gene; pre- and post-implantation defects	Infertility	111,112
Hepatocyte nuclear factor (HNF-1 $\alpha$ )(transcription factor 1; <i>Tcf1</i> )	Both	Infantile uterus; normal ovarian histology (F); vestigial vas deferens, seminal vesicles and prostate, impaired spermatogenesis, no mating behavior	Infertility	113

		(M)		
High mobility group box 2 ( <i>Hmgb2</i> )	Male	Sertoli and germ cell degeneration and immotile spermatozoa	Subfertility	114
Histone H2A family, member X ( <i>H2afx</i> )	Male	Pachytene stage arrest in spermatogenesis; defects in chromosome segregation and MLH1 foci formation	Infertile	115
Histone 3.3A gene ( <i>H3/3a</i> ) insertional mutation	Male	$\beta$ -gal gene trap insertion creates a hypomorphic allele; homozygous males have reduced copulatory activity and fewer matings result in pregnancy	Subfertility	116
Homeobox A10 ( <i>Hoxa10</i> )	Both	Variable infertility; males have cryptorchidism and females have frequent embryo loss prior to implantation	Progressive infertility (M); subfertility (F)	117
Homeobox A11 ( <i>Hoxa11</i> )	Both	Females have uterine defects; males have malformed vas deferens and undescended testes	Infertility	118
<i>Hrb</i> (HIV-1 Rev binding protein) (RAB/Rip)	Male	Round-headed spermatozoa lack an acrosome (globozoospermia)	Infertility	119
Inhibin $\alpha$ ( <i>Inha</i> )	Both	Granulosa/Sertoli tumors, gonadotropin hormone-dependent	Infertility (F) Secondary infertility (M)	120,121
Inositol polyphosphate-5-phosphatase ( <i>Inpp5b</i> )	Male	Sperm have reduced motility and reduced ability to fertilize eggs; defects in fertilin $\beta$ processing	Infertility	122
Insulin-like growth factor 1 ( <i>Igf1</i> )	Both	Hypogonadal and infertile; disrupted spermatogenesis and vestigial ductal system, defects in mating behavior (M); impaired antral follicle formation (F)	Infertility	123
Insulin-like growth factor 2 receptor ( <i>Igf2r</i> ); T-associated maternal effect ( <i>Tme</i> ) mutation	Female (Hetero)	Mutation of maternal allele in pups causes developmental defects and embryonic/perinatal death	Lethality; maternal effect	124
Insulin-like hormone 3 ( <i>Ins13</i> )	Both	Bilateral cryptorchidism results in abnormal spermatogenesis in males; female subfertility associated with irregular estrous cycles	Subfertility	125
Insulin receptor substrate 2 ( <i>Irs2</i> )	Female	Small, anovulatory ovaries with reduced numbers of follicles	Infertility	126
Interleukin 11 ( <i>Il11</i> )	Female	Compromised implantation and decidualization	Infertility	127
JunD ( <i>Jund1</i> )	Male	Anomalous hormone levels and sperm structural defects	Infertility	128
Kit ligand ( <i>Kitl</i> )	Both	<i>steel</i> defect mutation causes defect in PGIC migration/survival; <i>pandu</i>	Infertility	129,130

Kit receptor ( <i>Kit</i> )	Both	mutation causes blocks in folliculogenesis in females <i>White spotting</i> null mutation causes PGC defects	Infertility	131
Leptin ( <i>Lep. ob/ob</i> ) mutant	Both	Obese and infertile with hypogonadotropic hypogonadism	Infertility	132,133
Leptin receptor ( <i>Lep. db/db</i> ) mutant	Both	Obese and infertile with hypogonadotropic hypogonadism	Infertility	134
Leukemia inhibitory factor ( <i>Lif</i> )	Female	Failed implantation	Infertility	135
<i>Limk2</i> (LIM motif-containing protein kinase 2)	Male	Degeneration of spermatogenic cells in the seminiferous tubules; increased apoptosis		136
Lipase, hormone sensitive (HSL) ( <i>Lipe</i> )	Male	Multiple abnormalities in spermatogenesis	Infertility	137,138
Luteinizing Hormone Receptor ( <i>Lhcgr</i> )	Both	Underdeveloped sex organs and infertility in both males and females; spermatogenesis arrested at round spermatid stage; preantral folliculogenesis block	Infertility	139,140
<i>Man2a2</i> ( $\alpha$ -mannosidase IIx)	Male	Defect in adherence of spermatogenic cells to Sertoli cells; germ cells prematurely released from the testis	Mostly infertile	141
<i>Mater</i> (maternal antigen that embryos require)	Female	Development beyond the two-cell stage is blocked; Maternal effect gene	Infertility	142
<i>Mlh1</i> (MutL homologue 1)	Both	Meiotic arrest and genomic instability	Infertility	143,144
<i>Mos</i> (Moloney sarcoma oncogene)	Female	Parthenogenetic activation, cysts and teratomas	Subfertility	145,146
<i>Msh4</i> (MutS homologue 4)	Both	Prophase I meiotic defects apparent at the zygotene/pachytene stage; germ cells lost within a few days post-partum	Infertility	147
<i>Msh5</i> (MutS homologue 5)	Both	Zygotene/pachytene meiotic defects with aberrant chromosome synapsis and apoptosis	Infertility	148,149
Microtubule-associated protein ( <i>Map7</i> )(E-MAP-115) insertional mutation	Male	Abnormal microtubules in germ cells and Sertoli cells	Infertility	150
<i>More</i> (microorchidia) insertional mutation	Male	Early arrest in meiosis and germ cell apoptosis	Infertility	151
<i>Mybl1</i> ( $\Delta$ -myb) myeloblastosis oncogene-like 1	Male	Germ cell meiotic arrest at the pachytene stage	Infertility	152
$\text{Na}^{+}/\text{K}^{+}/2\text{Cl}^{-}$ cotransporter (NKCC1) solute carrier family 12, member 2 ( <i>Slc12a2</i> )	Male	Low spermatid counts and compromised sperm transport	Infertility	153
Neuronal Helix-loop-Helix 2 ( <i>Nhlh2</i> )	Both	Males are infertile and hypogonadal; females are fertile when reared with	Infertility	154



		males		
Neuronal insulin receptor (NIR)	Both	Hypothalamic hypogonadism; impaired spermatogenesis and follicle maturation	Infertility	155
Nitric oxide synthase 3, endothelial cell ( <i>Nos3</i> ; <i>eNos</i> )	Female	Compromised ovulation, delayed meiotic progression from metaphase I	Subfertility	156
Nuclear receptor co-activator ( <i>Ncoal</i> ); steroid receptor coactivator-1 ( <i>SRC1</i> )	Both	Decreased responsiveness to steroid hormones in uterus, mammary glands (F), testes and prostate (M)	Fertile	157
Nuclear receptor co-repressor RIP40 ( <i>Nrip1</i> )	Female	Ovulation defect; ovaries accumulate luteinized, unruptured follicles	Infertility	158
Nuclear receptor subfamily 5, group A, member 1 ( <i>Nr5a1</i> ); Steroidogenic factor-1 ( <i>SF-1</i> )	Both	Gonadal agenesis in both sexes	Lethal	159
<i>Otx1</i> (orthodenticle homolog 1)	Both	Prepubescent dwarfism and hypogonadism; progressive recovery of follicular development and sperm development and fertility	Delayed fertility	160
Ovo	Male	Reduced fertility and underdeveloped genitalia	Subfertility	161
P2X1 receptor ( <i>P2rx1</i> )	Male	Oligospermia and defective vas deferens contraction	Infertility	162
<i>p18<sup>Intc</sup></i> ( <i>Cdkn2c</i> )	Male	Leydig cell hyperplasia and reduced testosterone production	Fertile	163
<i>p19<sup>Intc</sup></i> ( <i>Cdkn2d</i> )	Male	Testicular atrophy and germ cell apoptosis	Fertile	164
<i>p27<sup>Kip1</sup></i> ( <i>Cdkn1b</i> )	Both	CL differentiation failure and granulosa cell hyperplasia (F); males fertile with testicular hyperplasia	Infertility (F)	165,166
<i>p57<sup>Kip2</sup></i> ( <i>Cdkn1c</i> )	Both	Surviving mice show sexual immaturity	Mostly lethal	167
PAC <sub>1</sub> ; adenylate cyclase activating polypeptide 1 receptor 1 ( <i>Adcyap1r1</i> )	Female	Prolonged and irregular diestrous phase	Subfertility	168
PC4 (testicular germ cell protease) ( <i>Pcsk4</i> )	Male	Sperm have impaired fertilization ability	Infertility	169
Pentraxin 3 ( <i>Ptx3</i> )	Female	Defects in cumulus-oocyte complex (COC) integrity and ovulation	Subfertility	170
Phosphatidylinositol 3'-kinase ( <i>Pi3k</i> )	Male	Defects in proliferation and increased apoptosis of spermatogonia	Infertility	171
Phosphatidylinositol glycan, class A ( <i>Piga</i> )	Chimeric Male	Abnormal testes, epididymis and seminal vesicles	Variable Infertility; no allele transmission	172
<i>Pit1</i> (pituitary specific transcription factor 1)	Both	<i>Snell</i> dwarf mice have multiple anterior pituitary hormone deficiencies and	Infertility	173

Polyomavirus enhancer activator 3 ( <i>Pea3</i> )	Male	hypogonadism Normal mating behavior, but males do not set plugs or release sperm	Infertility	174
Postmeiotic segregation increased 2 ( <i>Pms2</i> )	Both	Abnormal chromosome synapsis in meiosis (M); female knockout zygotes have microsatellite instability in both maternal and paternal genomes; Maternal effect gene	Infertility (M)	175,176
Progesterone receptor ( <i>Pgr</i> )	Female	Defects in ovulation, implantation, sexual behavior, and mammary gland development	Infertility	177
Prolactin ( <i>Prl</i> )	Female	Females are infertile with irregular estrus cycles	Infertility	178
Prolactin receptor ( <i>Prlr</i> )	Both	Compromised ovulation, fertilization and preimplantation development in knockouts (F); defects in maternal behavior in knockouts and heterozygotes (F); variable infertility and subfertility in males	Infertility (F); Subfertility (M)	179,180
<i>Prop1</i> (paired like homeodomain factor 1; prophet of <i>Pit1</i> )	Both	<i>Ames</i> dwarf mice have multiple anterior pituitary hormone deficiencies and hypogonadism	Infertility	181
Prostaglandin E2 EP2 receptor ( <i>Ptger2</i> )	Female	Decreased fertilization and defects in cumulus expansion	Subfertility	182-184
Prostaglandin F receptor ( <i>Ptgifr</i> )	Female	Females do not undergo parturition; failed luteolysis	Infertility	185
Protamine 1 ( <i>Prm1</i> )	Chimeric Male	Protamine haploinsufficiency; abnormal spermatogenesis	Infertility	186
Protamine 2 ( <i>Prm2</i> )	Chimeric Male	Protamine haploinsufficiency; abnormal spermatogenesis	Infertility	186
Protease inhibitor protease nexin-1 (PN-1) knockout ( <i>Serpine2</i> )	Male	Abnormal seminal vesicle morphology and altered semen protein composition	Subfertility	187
Protein kinase A, catalytic subunit $\alpha$ ( <i>Prkaca</i> )	Male	Most mice die; few viable mice have sperm motility defects	Mostly lethal	188
Protein phosphatase 1 catalytic subunit $\gamma$ ( <i>Ppp1cc</i> )	Male	Defects in spermiogenesis	Infertility	189
Protein phosphatase 1 regulatory subunit 1B ( <i>Ppp1r1b</i> ) (DARPP-32)	Female	Knockouts exhibited defects in progesterone facilitated sexual receptivity	Not reported	190
Puromycin-sensitive aminopeptidase ( <i>Psa</i> )	Female	Lack of CL formation and prolactin production cause early pregnancy loss	Infertility	191
Retinoic Acid Receptor alpha ( <i>Rara</i> )	Male	Complete arrest and degeneration or germ cell depletion	Infertility	192
Retinoic acid receptor $\gamma$ ( <i>Rarg</i> )	Male	Squamous metaplasia of the seminal vesicles and prostate	Infertility	193
Retinoid X receptors ( <i>Rxrb</i> )	Male	Germ cell maturation defects and tubular degeneration	Infertility	194
<i>Rosl</i> (c-ros)	Male	Sperm motility defects	Infertility	195,196

protoncogene)				
Scavenger receptor, class B1 ( <i>Srh1</i> )	Female	Defects in oocyte maturation and early embryo development due to abnormal lipoprotein metabolism	Infertility	197-199
<i>Serpina5</i> (Serine proteinase inhibitor A5; Protein C inhibitor)	Male	Sertoli cell destruction	Infertility	200
SH2-B	Both	Males have small testes and reduced sperm count; females have small, anovulatory ovaries with reduced numbers of developing follicles	Subfertility (M) Infertility (F)	201
Smad1 (MAD homolog 1; <i>Madh1</i> )	Both	Developing embryos lose PGCs	Lethal	202
Smad5 (MAD homolog 5; <i>Madh5</i> )	Both	Developing embryos lose PGCs	Lethal	203
Sp4 trans-acting transcription factor ( <i>Sp4</i> )	Male	Defects in reproductive behavior	Infertility	204
<i>Spam1</i> (sperm adhesion molecule) mutations	Female	Sperm defects in hyaluronic-acid binding	Subfertility	205
Sperm-1	Male	Defect in haploid sperm function	Subfertility	206
Sperm mitochondrion-associated cysteine-rich protein (SMCP)	Male	Defects in sperm motility and migration into the oviduct; defects in fertilization	Subfertility and Infertility	207
Spermatid perinuclear RNA-binding protein ( <i>Spnr</i> ) insertional mutation	Male	Defects in seminiferous epithelium and spermatogenesis	Subfertility	208
SPO11 homolog ( <i>Spo11</i> )	Both	Defects in meiosis; oocytes lost soon after birth	Infertility	209,210
Steroid 5 $\alpha$ -reductase type 1 ( <i>Srd5a1</i> )	Female	Defects in parturition	Infertility	211,212
Steroidogenic acute regulatory protein ( <i>Star</i> )	Both	Males have female external genitalia; both sexes die of adrenocortical insufficiency	Lethal	213
<i>Stxr</i> (phosphoserine/threonine/tyrosine interaction protein)	Male	Defects in round and elongating spermatid development	Infertility	214
Superoxide dismutase 1 ( <i>Sod1</i> )	Female	Folliculogenesis defect; failure to maintain pregnancy	Subfertility	215,216
<i>Sycp3</i> (synaptonemal complex protein 3)	Both	Defects in chromosome synapsis during meiosis; germ cell apoptosis in males; embryonic loss in females due to aneuploidy	Infertility (M) Subfertility (F)	217,218
<i>Taf4b</i> (TAF4B RNA polymerase II, TATA box binding protein-associated factor; TAFII105)	Female	Defects in follicular development, oocyte maturation/fertilization	Infertility	219
TATA-binding protein-like protein ( <i>Tlp</i> )	Male	Post-meiotic spermiogenesis block (defective acrosome formation in early	Infertility	220,221

TRF2) Telomerase reverse transcriptase ( <i>Tert</i> )	Both	stage spermatids) Progressive infertility in both sexes; females have few oocytes and uterine abnormalities	Progressive Infertility	222
<i>Theg</i> ( <i>kisimo</i> ) (Transgene integration)	Male	Abnormal elongated spermatids; asthenospermia	Infertility	223
Thyroid stimulating hormone $\beta$ ( <i>Tshb</i> ; <i>hyt/hyt</i> ) mutant	Female	Hypothyroid; females show continuous dioestrus, and poor response to gonadotropin-induced superovulation	Infertility	224
<i>Tial1</i> (cytotoxic granule-associated RNA binding protein-like 1)	Both	PGCs lost by E13.5	Infertility	225
<i>Tnp1</i> (transition protein 1)	Male	Abnormal chromosome condensation, sperm motility	Subfertility	226
<i>Tnp2</i> (transition protein 2)	Male	Abnormal chromosome condensation	Subfertility	227
Tumor necrosis factor type I receptor ( <i>Tnfrsf1a</i> )	Female	Enhanced prepubertal response to gonadotropins; early ovarian senescence	Subfertility	228
<i>Ube2h</i> (E2B ubiquitin-conjugating enzyme; HR6B)	Male	Alterations in sperm chromatin structure, an incomplete meiotic arrest, abnormal sperm morphology	Infertility	229
Ubiquitin-like DNA repair gene HR23B ( <i>Rad23b</i> )	Male	Most knockouts die during development or shortly after birth; surviving mice have multiple abnormalities and male sterility	Variable lethality; Infertility	230
Ubiquitin protein ligase E3A ( <i>Ube3a</i> ; E6-AP ubiquitin protein ligase)	Both	Testicular hypoplasia, defects in spermatogenesis and prostate gland development (M); ovarian hypoplasia, defects in ovulation and uterine development (F)	Subfertility	231
Ubiquitin protein ligase seven in absentia 1A ( <i>Siuh1a</i> )	Male	Block in spermatogenesis and germ cell apoptosis; failure to complete transition to telophase of meiosis I	Partially lethal; Infertility	232
VASA homolog ( <i>Ddx4</i> ; DEAD box polypeptide 4)	Male	Defective proliferation/differentiation of PGCs	Infertility	233
Vitamin D receptor ( <i>Vdr</i> ) knockout	Both	Defects in estrogen biosynthesis in males and females; elevated serum gonadotropins	Infertility	234,235
Voltage-dependent Anion Channel Type 3 ( <i>Vdac3</i> )	Male	Immotile sperm; axonemal defects with sperm maturation	Infertility	236
Wilms tumor homolog ( <i>Wt1</i> )	Both	Gonadal agenesis	Lethal	237
<i>Wip1</i> (p53-induced phosphatase)	Male	Runting and testicular atrophy	Subfertility	238
Wingless-related MM'V integration site 4 ( <i>Wnt4</i> )	Female	Ovaries depleted of oocytes; Müllerian ducts do not form	Infertility	239

<i>Wnt7a</i>	Both	Females show abnormal development of oviducts and uterus; males do not have Müllerian duct regression	Infertility	240
<i>Zfx</i> (Zinc finger protein X-linked)	Both	Reduced germ cell numbers; males have reduced sperm, but are fertile; females subfertile	Subfertility (F)	241
Zona pellucida protein 1 ( <i>Zp1</i> )	Female	Defects in fertilization	Subfertility	242
<i>Zp2</i>	Female	Fragile oocytes with defects in developmental competence	Infertility	243
<i>Zp3</i>	Female	Fragile oocytes	Infertility	244,245

## REFERENCES

- Adham, I.M., Nayernia, K. & Engel, W. Spermatozoa lacking acrosin protein show delayed fertilization. *Mol Reprod Dev* **46**, 370-6 (1997).
- Matzuk, M.M., Kumar, T.R. & Bradley, A. Different phenotypes for mice deficient in either activins or activin receptor type II. *Nature* **374**, 356-360 (1995).
- Vassalli, A., Matzuk, M.M., Gardner, H.A.R., Lee, K.-F. & Jaenisch, R. Activin/inhibin  $\beta$ B subunit gene disruption leads to defects in eyelid development and female reproduction. *Genes & Development* **8**, 414-427 (1994).
- Cho, Y.Y. et al. Abnormal uterus with polycysts, accumulation of uterine prostaglandins, and reduced fertility in mice heterozygous for acyl-CoA synthetase 4 deficiency. *Biochem Biophys Res Commun* **284**, 993-7 (2001).
- Shindo, T. et al. ADAMTS-1: a metalloproteinase-disintegrin essential for normal growth, fertility, and organ morphology and function. *J Clin Invest* **105**, 1345-52 (2000).
- Li, S.W. et al. Transgenic mice with inactive alleles for procollagen N-proteinase (ADAMTS-2) develop fragile skin and male sterility. *Biochem J* **355**, 271-8 (2001).
- Schurmann, A. et al. Reduced sperm count and normal fertility in male mice with targeted disruption of the ADP-ribosylation factor-like 4 (*Arl4*) gene. *Mol Cell Biol* **22**, 2761-8 (2002).
- Sato, H. et al. Impaired fertility in female mice lacking urinary trypsin inhibitor. *Biochem Biophys Res Commun* **281**, 1154-60 (2001).
- Krege, J.H. et al. Male-female differences in fertility and blood pressure in ACE- deficient mice. *Nature* **375**, 146-8 (1995).
- Charest, N.J. et al. A frameshift mutation destabilizes androgen receptor messenger RNA in the Tfm mouse. *Mol Endocrinol* **5**, 573-81 (1991).
- Behringer, R.R., Finegold, M.J. & Cate, R.L. Müllerian-inhibiting substance function during mammalian sexual development. *Cell* **79**, 415-425 (1994).
- Durlinger, A.L. et al. Control of primordial follicle recruitment by anti-Müllerian hormone in the mouse ovary. *Endocrinology* **140**, 5789-96 (1999).
- Mishina, Y. et al. Genetic analysis of the Müllerian-inhibiting substance signal transduction pathway in mammalian sexual differentiation. *Genes and Development* **10**, 2577-2587 (1996).
- Honarpour, N. et al. Adult Apaf-1-deficient mice exhibit male infertility. *Dev Biol* **218**, 248-258 (2000).
- Huang, L.S., Voyiatzakis, H., Chen, H.L., Rubin, E.M. & Gordon, J.W. A novel functional role for apolipoprotein B in male infertility in heterozygous apolipoprotein B knockout mice. *Proc Natl Acad Sci USA* **93**, 10903-7 (1996).
- Benedict, J.C., Lin, T.M., Locflier, I.K., Peterson, R.E. & Flaws, J.A. Physiological role of the aryl hydrocarbon receptor in mouse ovary development. *Toxicol Sci* **56**, 382-8. (2000).
- Abbott, B.D. et al. Adverse reproductive outcomes in the transgenic Ah receptor-deficient mouse. *Toxicol Appl Pharmacol* **155**, 62-70 (1999).

18. Barlow, C. et al. *Atm* deficiency results in severe meiotic disruption as early as leptotema of prophase I. *Development* **125**, 4007-4017 (1998).
19. Xu, Y. et al. Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. *Genes & Development* **10**, 2411-2422 (1996).
20. Christiansen-Weber, T.A. et al. Functional loss of ABCA1 in mice causes severe placental malformation, aberrant lipid distribution, and kidney glomerulonephritis as well as high-density lipoprotein cholesterol deficiency. *Am J Pathol* **157**, 1017-29 (2000).
21. Kuno, N. et al. Female sterility in mice lacking the *basigin* gene, which encodes a transmembrane glycoprotein belonging to the immunoglobulin superfamily. *Federation of European Biochemical Sciences* **425**, 191-194 (1998).
22. Igakura, T. et al. A null mutation in basigin, an immunoglobulin superfamily member, indicates its important roles in peri-implantation development and spermatogenesis. *Dev Biol* **194**, 152-165 (1998).
23. Knudson, C.M., Tung, K.S.K., Tourtellotte, W.G., Brown, G.A.J. & Korsmeyer, A.J. Bax-deficient mice with lymphoid hyperplasia and male germ cell death. *Science* **270**, 96-99 (1995).
24. Perez, G.I. et al. Prolongation of ovarian lifespan into advanced chronological age by *Bax*-deficiency. *Nature Genetics* **21**, 200-203 (1999).
25. Ratts, V.S., Flaws, J.A., Kolp, R., Sorenson, C.M. & Tilly, J.L. Ablation of *bcl-2* gene expression decreases the numbers of oocytes and primordial follicles established in the post-natal female mouse gonad. *Endocrinology* **136**, 3665-3668 (1995).
26. Kojima, S. et al. Testicular germ cell apoptosis in *Bcl6*-deficient mice. *Development* **128**, 57-65 (2001).
27. Rucker, E.B., 3rd et al. *Bcl-x* and *Bax* regulate mouse primordial germ cell survival and apoptosis during embryogenesis. *Mol Endocrinol* **14**, 1038-52 (2000).
28. Ross, A.J. et al. Testicular degeneration in *Bclw*-deficient mice. *Nat Genet* **18**, 251-6 (1998).
29. Lawson, K.A. et al. *Bmp4* is required for the generation of primordial germ cells in the mouse embryo. *Genes Dev* **13**, 424-436 (1999).
30. Zhao, G.-Q., Liaw, L. & Hogan, B.L.M. Bone morphogenetic protein 8A plays a role in the maintenance of spermatogenesis and the integrity of the epididymis. *Development* **125**, 1103-1112 (1998).
31. Zhao, G.-Q., Deng, K., Labosky, P.A., Liaw, L. & Hogan, B.L.M. The gene encoding bone morphogenetic protein 8B is required for the initiation and maintenance of spermatogenesis in the mouse. *Genes Dev* **10**, 1657-1669 (1996).
32. Yan, C. et al. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. *Mol Endocrinol* **15**, 854-866 (2001).
33. Yi, S.F. et al. The type I BMP receptor *Bmpr1B* is essential for female reproductive function. *Proc Natl Acad Sci USA* **98**, 7994-9 (2001).
34. Ikawa, M. et al. The putative chaperone calnexin is required for sperm fertility. *Nature* **387**, 607-611 (1997).
35. Wu, J., Gonzalez-Robayna, I., Richards, J. & Means, A. Female fertility is reduced in mice lacking *Ca2+/calmodulin-dependent protein kinase IV*. *Endocrinology* **141**, 4777-83 (2000).
36. Blendy, J.A., Kaestner, K.H., Weinbauer, G.F., Nieschlag, E. & Schutz, G. Severe impairment of spermatogenesis in mice lacking the *CREM* gene. *Nature* **380**, 162-5 (1996).
37. Nantel, F. et al. Spermiogenesis deficiency and germ-cell apoptosis in *CREM*-mutant mice. *Nature* **380**, 159-162 (1996).
38. Jin, S.L., Richard, F.J., Kuo, W.P., D'Ercole, A.J. & Conti, M. Impaired growth and fertility of cAMP-specific phosphodiesterase *PDE4D*-deficient mice. *Proc Natl Acad Sci USA* **96**, 11998-2003 (1999).
39. Xu, X., Toselli, P.A., Russell, L.D. & Seldin, D.C. Globozoospermia in mice lacking the casein kinase II alpha catalytic subunit. *Nat Genet* **23**, 118-121 (1999).

40. Bergeron, I., et al. Defects in regulation of apoptosis in caspase-2-deficient mice. *Genes & Development* **12**, 1304-1314 (1998).
41. Ren, D. et al. A sperm ion channel required for sperm motility and male fertility. *Nature* **413**, 603-9 (2001).
42. Le Naour, F., Rubinstein, E., Jasmin, C., Prenant, M. & Boucheix, C. Severely reduced female fertility in CD9-deficient mice. *Science* **287**, 319-21 (2000).
43. Lincoln, A.J. et al. Cdc25b phosphatase is required for resumption of meiosis during oocyte maturation. *Nat Genet* **30**, 446-9 (2002).
44. Hudson, D.F. et al. Centromere protein B null mice are mitotically and meiotically normal but have lower body and testis weights. *J Cell Biol* **141**, 309-319 (1998).
45. Fowler, K.J. et al. Uterine dysfunction and genetic modifiers in centromere protein B- deficient mice. *Genome Res* **10**, 30-41 (2000).
46. Sierneck, E., Tessarollo, L. & Johnson, P.F. An essential role for C/EBP $\beta$  in female reproduction. *Genes & Development* **11**, 2153-2162 (1997).
47. Gow, A. et al. CNS myelin and sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. *Cell* **99**, 649-659 (1999).
48. Cohen, P.E., Zhu, L. & Pollard, J.W. Absence of colony stimulating factor-1 in osteopetrotic (*csf1<sup>op</sup>/csf1<sup>op</sup>*) mice disrupts estrous cycles and ovulation. *Biology of Reproduction* **56**, 110-118 (1997).
49. Robertson, S.A., Roberts, C.T., Farr, K.L., Dunn, A.R. & Seamark, R.F. Fertility impairment in granulocyte-macrophage colony-stimulating factor-deficient mice. *Biol Reprod* **60**, 251-61 (1999).
50. Simon, A.M., Goodenough, D.A., Li, E. & Paul, D.L. Female infertility in mice lacking connexin 37. *Nature* **385**, 525-529 (1997).
51. Juneja, S.C., Barr, K.J., Enders, G.C. & Kidder, G.M. Defects in the germ line and gonads of mice lacking connexin43. *Biol Reprod* **60**, 1263-1270 (1999).
52. Tay, J. & Richter, J.D. Germ cell differentiation and synaptonemal complex formation are disrupted in CPEB knockout mice. *Dev Cell* **1**, 201-13 (2001).
53. Luong, M. et al. Genetic ablation of the CDP/Cux protein C-terminus results in hair cycle defects and reduced male fertility. *Mol Cell Biol* **22**, 1424-37 (2002).
54. Liu, D. et al. Cyclin A1 is required for meiosis in the male mouse. *Nature Genetics* **20**, 377-388 (1998).
55. Sicinski, P. et al. Cyclin D2 is an FSH-responsive gene involved in gonadal cell proliferation and oncogenesis. *Nature* **384**, 470-474 (1996).
56. Ranc, S.G. et al. Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in  $\beta$ -islet cell hyperplasia. *Nature Genetics* **22**, 44-52 (1999).
57. Dinchuk, J.E. et al. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature* **378**, 406-409 (1995).
58. Lim, H. et al. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* **91**, 197-208 (1997).
59. Ilu, M.C. et al. Steroid deficiency syndromes in mice with targeted disruption of CYP11A1. *Molecular Endocrinology* **16**, 1943-50 (2002).
60. Robertson, K.M. et al. Impairment of spermatogenesis in mice lacking a functional aromatase (*cyp 19*) gene. *Proc Natl Acad Sci USA* **96**, 7986-7991 (1999).
61. Britt, K.L. et al. An age-related ovarian phenotype in mice with targeted disruption of the *Cyp19* (aromatase) gene. *Endocrinology*, **141**, 2614-2623 (2000).
62. Robertson, K.M., Simpson, E.R., Lacham-Kaplan, O. & Jones, M.E. Characterization of the fertility of male aromatase knockout mice. *J Androl* **22**, 825-30 (2001).
63. Panda, D.K. et al. Targeted ablation of the 25-hydroxyvitamin D 1 $\alpha$  -hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. *Proc Natl Acad Sci U S A* **98**, 7498-503 (2001).

64. Shamsadin, R. et al. Male mice deficient for germ-cell cyritestin are infertile. *Biol Reprod* **61**, 1445-1451 (1999).
65. Nishimura, H., Cho, C., Branciforte, D.R., Myles, D.G. & Primakoff, P. Analysis of loss of adhesive function in sperm lacking cyritestin or fertilin beta. *Dev Biol* **233**, 204-13 (2001).
66. Yu, R.N., Ito, M., Saunders, T.L., Camper, S.A. & Jameson, J. Role of Ahch in gonadal development and gametogenesis. *Nat Genet* **20**, 353-357 (1998).
67. Ruggiu, M. et al. The mouse *Dazl* gene encodes a cytoplasmic protein essential for gametogenesis. *Nature* **389**, 73-76 (1997).
68. Bitgood, M.J., Shen, L. & McMahon, A.P. Sertoli cell signaling by Desert hedgehog regulates the male germline. *Curr Biol* **6**, 298-304 (1996).
69. Clark, A.M., Garland, K.K. & Russell, L.D. Desert hedgehog (Dhh) gene is required in the mouse testis for formation of adult-type Leydig cells and normal development of peritubular cells and seminiferous tubules. *Biol Reprod* **63**, 1825-38 (2000).
70. Pittman, D.L. et al. Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for *Dmcl*, a germline-specific RecA homolog. *Molecular Cell* **1**, 697-705 (1998).
71. Yoshida, K. et al. The mouse RecA-like gene *Dmcl* is required for homologous chromosome synapsis during meiosis. *Molecular Cell* **1**, 707-718 (1998).
72. Howell, C.Y. et al. Genomic imprinting disrupted by a maternal effect mutation in the *Dnmt1* gene. *Cell* **104**, 829-38 (2001).
73. Kobayashi, Y. et al. Hydrocephalus, situs inversus, chronic sinusitis, and male infertility in DNA polymerase lambda-deficient mice: possible implication for the pathogenesis of immotile cilia syndrome. *Mol Cell Biol* **22**, 2769-76 (2002).
74. Raymond, C.S., Murphy, M.W., O'Sullivan, M.G., Bardwell, V.J. & Zarkower, D. *Dmrt1*, a gene related to worm and fly sexual regulators, is required for mammalian testis differentiation. *Genes Dev* **14**, 2587-95 (2000).
75. Neesen, J. et al. Disruption of an inner arm dynein heavy chain gene results in asthenozoospermia and reduced ciliary beat frequency. *Hum Mol Genet* **10**, 1117-28. (2001).
76. Topilko, P. et al. Multiple pituitary and ovarian defects in Krox-24 (NGFI-A, Egr-1)-targeted mice. *Mol Endocrinol* **12**, 107-122 (1998).
77. Lee, S.L. et al. Luteinizing hormone deficiency and female infertility in mice lacking the transcription factor NGFI-A (Egr-1). *Science* **273**, 1219-1221 (1996).
78. Tourtellotte, W.G., Nagarajan, R., Auyeung, A., Mueller, C. & Milbrandt, J. Infertility associated with incomplete spermatogenic arrest and oligozoospermia in *Egr4*-deficient mice. *Development* **126**, 5061-71 (1999).
79. Bessone, S. et al. EMK protein kinase-null mice: dwarfism and hypofertility associated with alterations in the somatotrope and prolactin pathways. *Dev Biol* **214**, 87-101 (1999).
80. Miyamoto, N., Yoshida, M., Kuratani, S., Matsuo, I. & Aizawa, S. Defects of urogenital development in mice lacking *Emx2*. *Development* **124**, 1653-1664 (1997).
81. Lubahn, D.B. et al. Alteration of reproductive function but not prenatal sexual development after inscircular disruption of the mouse estrogen receptor gene. *Proceedings of the National Academy of Sciences USA* **90**, 11162-11166 (1993).
82. Hess, R.A. et al. A role for oestrogens in the male reproductive system. *Nature* **390**, 509-12 (1997).
83. Ogawa, S. et al. Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. *Endocrinology* **139**, 5070-81 (1998).
84. Ogawa, S., Lubahn, D.B., Korach, K.S. & Pfaff, D.W. Behavioral effects of estrogen receptor gene disruption in male mice. *Proc Natl Acad Sci U S A* **94**, 1476-81 (1997).
85. Krege, J.H. et al. Generation and reproductive phenotypes of mice lacking estrogen receptor  $\beta$ . *Proc Natl Acad Sci USA* **95**, 15677-15682 (1998).
86. Cheng, N.C. et al. Mice with a targeted disruption of the Fanconi anemia homolog *Fanca*. *Hum Mol Genet* **9**, 1805-11 (2000).



87. Chen, M. et al. Inactivation of *Fac* in mice produces inducible chromosomal instability and reduced fertility reminiscent of Fanconi anaemia. *Nature Genetics* **12**, 448-451 (1996).
88. Whitney, M.A. et al. Germ cell defects and hematopoietic hypersensitivity to  $\gamma$ -interferon in mice with a targeted disruption of the fanconi anemia C gene. *Blood* **88**, 49-58 (1996).
89. Koomen, M. et al. Reduced fertility and hypersensitivity to mitomycin C characterize *Fancg/Xrcc9* null mice. *Hum Mol Genet* **11**, 273-281 (2002).
90. Cho, C. et al. Fertilization defects in sperm from mice lacking fertilin beta. *Science* **281**, 1857-1859 (1998).
91. Colvin, J.S., Green, R.P., Schmahl, J., Capel, B. & Ornitz, D.M. Male-to-female sex reversal in mice lacking fibroblast growth factor 9. *Cell* **104**, 875-89 (2001).
92. Soyol, S.M., Amleh, A. & Dean, J. FIG $\alpha$ , a germ cell-specific transcription factor required for ovarian follicle formation. *Development* **127**, 4645-54 (2000).
93. Kooy, R.F. et al. Transgenic mouse model for the fragile X syndrome. *Am J Med Genet* **64**, 241-5 (1996).
94. Kumar, T.R., Wang, Y., Lu, N. & Matzuk, M.M. Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. *Nature Genetics* **15**, 201-204 (1997).
95. Dierich, A. et al. Impairing follicle-stimulating hormone (FSH) signaling *in vivo*: Targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. *Proc Natl Acad Sci USA* **95**, 13612-13617 (1998).
96. Kuroda, M. et al. Male sterility and enhanced radiation sensitivity in *TSL (-/-)* mice. *EMBO J* **19**, 453-462 (2000).
97. Lu, Q., Hasty, P. & Shur, B.D. Targeted mutation in  $\beta$ 1,4-galactosyltransferase leads to pituitary insufficiency and neonatal lethality. *Developmental Biology* **181**, 257-267 (1997).
98. Lu, Q. & Shur, B.D. Sperm from  $\beta$ 1,4-galactosyltransferase-null mice are refractory to ZP3-induced acrosome reactions and penetrate the zona pellucida poorly. *Development* **124**, 4121-4131 (1997).
99. Kumar, T.R. et al. Reproductive defects in gamma-glutamyl transpeptidase-deficient mice. *Endocrinology* **141**, 4270-7 (2000).
100. Lieberman, M.W. et al. Growth retardation and cysteine deficiency in  $\gamma$ -glutamyl transpeptidase-deficient mice. *Proc Natl Acad Sci USA* **93**, 7923-7926 (1996).
101. Togawa, A. et al. Progressive impairment of kidneys and reproductive organs in mice lacking Rho GDI $\alpha$ . *Oncogene* **18**, 5373-5380 (1999).
102. Meng, X. et al. Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. *Science* **287**, 1489-1493 (2000).
103. Kendall, S.K., Samuelson, L.C., Saunders, T.L., Wood, R.I. & Camper, S.A. Targeted disruption of the pituitary glycoprotein hormone  $\alpha$ -subunit produces hypogonadal and hypothyroid mice. *Genes and Development* **9**, 2007-2019 (1995).
104. Overbeek, P.A. et al. A transgenic insertion causing cryptorchidism in mice. *Genesis* **30**, 26-35 (2001).
105. Settle, S. et al. The bmp family member *gdf7* is required for seminal vesicle growth, branching morphogenesis, and cytodifferentiation. *Dev Biol* **234**, 138-50 (2001).
106. Dong, J. et al. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature* **383**, 531-535 (1996).
107. Elvin, J.A., Yan, C., Wang, P., Nishimori, K. & Matzuk, M.M. Molecular characterization of the follicle defects in the growth differentiation factor-9-deficient ovary. *Molecular Endocrinology* **13**, 1018-1034 (1999).
108. Zhou, Y. et al. A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). *Proc Natl Acad Sci USA* **94**, 13215-20 (1997).
109. Leighton, P.A., Ingram, R.S., Eggenschwiler, J., Efstratiadis, A. & Tilghman, S.M. Disruption of imprinting caused by deletion of the *H19* gene region in mice. *Nature* **375**, 34-9 (1995).

110. Dix, D.J. et al. Targeted gene disruption of Hsp70-2 results in failed meiosis, germ cell apoptosis, and male infertility. *Proc Natl Acad Sci U S A* **93**, 3264-8 (1996).
111. Xiao, X. et al. HSF1 is required for extra-embryonic development, postnatal growth and protection during inflammatory responses in mice. *The EMBO Journal* **18**, 5943-5952 (1999).
112. Christians, E., Davis, A.A., Thomas, S.D. & Benjamin, I.J. Maternal effect of Hsf1 on reproductive success. *Nature* **407**, 693-4 (2000).
113. Lee, Y.H., Sauer, B. & Gonzalez, F.J. Laron dwarfism and non-insulin-dependent diabetes mellitus in the Hnf-1alpha knockout mouse. *Mol Cell Biol* **18**, 3059-68 (1998).
114. Ronfani, L. et al. Reduced fertility and spermatogenesis defects in mice lacking chromosomal protein Hmgb2. *Development* **128**, 1265-73 (2001).
115. Celeste, A. et al. Genomic instability in mice lacking histone H2AX. *Science* **296**, 922-7 (2002).
116. Couldrey, C., Carlton, M.B., Nolan, P.M., Colledge, W.H. & Evans, M.J. A retroviral gene trap insertion into the histone 3.3A gene causes partial neonatal lethality, stunted growth, neuromuscular deficits and male sub-fertility in transgenic mice. *Hum Mol Genet* **8**, 2489-95 (1999).
117. Satokata, I., Benson, G. & Maas, R. Sexually dimorphic sterility phenotypes in Hoxa10-deficient mice. *Nature* **374**, 460-463 (1995).
118. Hsieh-Li, H.M. et al. *Hoxa 11* structure, extensive antisense transcription, and function in male and female fertility. *Development* **121**, 1373-1385 (1995).
119. Kang-Decker, N., Mantchev, G.T., Juneja, S.C., McNiven, M.A. & van Deursen, J.M. Lack of acrosome formation in *Hrb*-deficient mice. *Science* **294**, 1531-3 (2001).
120. Matzuk, M.M., Finegold, M.J., Su, J.-G.J., Hsueh, A.J.W. & Bradley, A.  $\alpha$ -Inhibin is a tumor-suppressor gene with gonadal specificity in mice. *Nature* **360**, 313-319 (1992).
121. Kumar, T.R., Wang, Y. & Matzuk, M.M. Gonadotropins are essential modifier factors for gonadal tumor development in inhibin-deficient mice. *Endocrinology* **137**, 4210-4216 (1996).
122. Hellsten, E., Evans, J.P., Bernard, D.J., Janne, P.A. & Nussbaum, R.L. Disrupted sperm function and fertilin beta processing in mice deficient in the inositol polyphosphate 5-phosphatase *Inpp5b*. *Dev Biol* **240**, 641-53 (2001).
123. Baker, J. et al. Effects of an *Igf1* gene null mutation on mouse reproduction. *Molecular Endocrinology* **10**, 903-918 (1996).
124. Lau, M.M. et al. Loss of the imprinted IGF2/cation-independent mannose 6-phosphate receptor results in fetal overgrowth and perinatal lethality. *Genes Dev* **8**, 2953-63 (1994).
125. Nef, S. & Parada, L.F. Cryptorchidism in mice mutant for *Ins13*. *Nat Genet* **22**, 295-9 (1999).
126. Burks, D.J., Withers, D.J., Towery, H., Altamuro, S. & White, M.F. Disruption of IRS-2 causes infertility in female mice. in *The Endocrine Society's 81st Annual Meeting* **76** (The Endocrine Society, San Diego, CA, 1999).
127. Robb, L. et al. Infertility in female mice lacking the receptor for interleukin II is due to a defective uterine response to implantation. *Nature Medicine* **4**, 303-308 (1998).
128. Thepot, D. et al. Targeted disruption of the murine *junD* gene results in multiple defects in male reproductive function. *Development* **127**, 143-153 (2000).
129. Matsui, Y., Zsebo, K.M. & Hogan, B.L.M. Embryonic expression of a haematopoietic growth factor encoded by the *Sf* locus and the ligand for *c-kit*. *Nature* **347**, 667-669 (1990).
130. Beechey, C.V., Loutit, J.F. & Searle, A.G. Panda, a new steel allele. *Mouse News Letter* **74**, 92 (1986).
131. Manova, K., Nocka, K., Besmer, P. & Bachvarova, R.F. Gonadal expression of *c-kit* encoded at the *W* locus of the mouse. *Development* **110**, 1057-1069 (1990).
132. Swerdloff, R.S., Batt, R.A. & Bray, G.A. Reproductive hormonal function in the genetically obese (*ob/ob*) mouse. *Endocrinology* **98**, 1359-64 (1976).
133. Swerdloff, R.S. et al. The hypothalamic-pituitary axis in genetically obese (*ob/ob*) mice: response to luteinizing hormone-releasing hormone. *Endocrinology* **103**, 542-7 (1978).
134. Chua, S.C., Jr. et al. Phenotypes of mouse diabetes and rat fatty due to mutations in the *OIS* (leptin) receptor. *Science* **271**, 994-6 (1996).

135. Stewart, C.L., et al. Blastocyst implantation depends on maternal expression of leukemia inhibitory-function. *Nature* **359**, 76-79 (1992).
136. Takahashi, H., Koshimizu, U., Miyazaki, J. & Nakamura, T. Impaired spermatogenic ability of testicular germ cells in mice deficient in the LIM-kinase 2 gene. *Dev Biol* **241**, 259-72 (2002).
137. Chung, S. et al. Infertility and testicular defects in hormone-sensitive lipase-deficient mice. *Endocrinology* **142**, 4272-81 (2001).
138. Osuga, J. et al. Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. *Proc Natl Acad Sci USA* **97**, 787-792 (2000).
139. Zhang, F.P., Poutanen, M., Wilbertz, J. & Huhtaniemi, I. Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. *Mol Endocrinol* **15**, 172-83 (2001).
140. Lei, Z.M. et al. Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. *Mol Endocrinol* **15**, 184-200 (2001).
141. Akama, T.O. et al. Germ cell survival through carbohydrate-mediated interaction with Sertoli cells. *Science* **295**, 124-7 (2002).
142. Tong, Z.B. et al. Mater, a maternal effect gene required for early embryonic development in mice. *Nat Genet* **26**, 267-8 (2000).
143. Edelmann, W. et al. Meiotic pachytene arrest in MLH1-deficient mice. *Cell* **85**, 1125-1134 (1996).
144. Baker, S.M. et al. Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nature Genetics* **13**, 336-341 (1996).
145. Colledge, W.H., Carlton, M.B., Udy, G.B. & Evans, M.J. Disruption of *c-mos* causes parthenogenetic development of unfertilized mouse eggs. *Nature* **370**, 65-68 (1994).
146. Hashimoto, N. et al. Parthenogenetic activation of oocytes in *c-mos*-deficient mice. *Nature* **370**, 68-71 (1994).
147. Kneitz, B. et al. MutS homolog 4 localization to meiotic chromosomes is required for chromosome pairing during meiosis in male and female mice. *Genes Dev* **14**, 1085-97 (2000).
148. deVries, S.S. et al. Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. *Genes Dev* **13**, 523-531 (1999).
149. Edelmann, W. et al. Mammalian MutS homologue 5 is required for chromosome pairing in meiosis. *Nat Genet* **21**, 123-127 (1999).
150. Komada, M., McLean, D.J., Griswold, M.D., Russell, L.D. & Soriano, P. E-MAP-115, encoding a microtubule-associated protein, is a retinoic acid-inducible gene required for spermatogenesis. *Genes Dev* **14**, 1332-42 (2000).
151. Watson, M.L. et al. Identification of more (microorchidia), a mutation that results in arrest of spermatogenesis at an early meiotic stage in the mouse. *Proc Natl Acad Sci USA* **95**, 14361-6 (1998).
152. Toscani, A. et al. Arrest of spermatogenesis and defective breast development in mice lacking *A-myb*. *Nature* **386**, 713-717 (1997).
153. Pace, A.J. et al. Failure of spermatogenesis in mouse lines deficient in the Na(+)-K(+)-2Cl(-) cotransporter. *J Clin Invest* **105**, 441-450 (2000).
154. Good, D.J. et al. Hypogonadism and obesity in mice with a targeted deletion of the *Nhlh2* gene. *Nature Genetics* **15**, 397-401 (1997).
155. Bruning, J.C. et al. Role of brain insulin receptor in control of body weight and reproduction. *Science* **289**, 2122-5 (2000).
156. Jablonka-Shariff, A. & Olson, L.M. The role of nitric oxide in oocyte meiotic maturation and ovulation: meiotic abnormalities of endothelial nitric oxide synthase knock-out mouse oocytes. *Endocrinology* **139**, 2944-54 (1998).
157. Xu, J. et al. Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. *Science* **279**, 1922-5 (1998).
158. White, R. et al. The nuclear receptor co-repressor nr1p (RIP140) is essential for female fertility. *Nat Med* **6**, 1368-74 (2000).

159. Luo, X., Ikeda, Y. & Parker, K.L. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell* **77**, 481-490 (1994).
160. Acampora, D. et al. Transient dwarfism and hypogonadism in mice lacking *Otx1* reveal prepubescent stage-specific control of pituitary levels of GH, FSH and LH. *Development* **125**, 1229-39 (1998).
161. Dai, X. et al. The ovo gene required for cuticle formation and oogenesis in flies is involved in hair formation and spermatogenesis in mice. *Genes Dev* **12**, 3452-3463 (1998).
162. Mulryan, K. et al. Reduced vas deferens contraction and male infertility in mice lacking P2X1 receptors. *Nature* **403**, 86-89 (2000).
163. Zindy, F. et al. Control of spermatogenesis in mice by the cyclin D-dependent kinase inhibitors p18(Ink4c) and p19(Ink4d). *Mol Cell Biol* **21**, 3244-55 (2001).
164. Zindy, F., Deursen, J.v., Grosveld, G., Sherr, C.J. & Roussel, M.F. INK4d-deficient mice are fertile despite testicular atrophy. *Mol Cell Biol* **20**, 372-378 (2000).
165. Nakayama, K. et al. Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. *Cell* **85**, 707-720 (1996).
166. Fero, M.L. et al. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. *Cell* **85**, 733-44 (1996).
167. Takahashi, K., Kobayashi, T. & Kanayama, N. p57(Kip2) regulates the proper development of labyrinthine and spongiotrophoblasts. *Mol Hum Reprod* **6**, 1019-25 (2000).
168. Jamen, F. et al. PAC1 null females display decreased fertility. *Ann N Y Acad Sci* **921**, 400-4 (2000).
169. Mbikay, M. et al. Impaired fertility in mice deficient for the testicular germ-cell protease PC4. *Proc Natl Acad Sci U S A* **94**, 6842-6 (1997).
170. Varani, S. et al. Knockout of pentraxin 3, a downstream target of growth differentiation factor-9, causes female subfertility. *Molecular Endocrinology* **16**, 1154-1167 (2002).
171. Blume-Jensen, P. et al. Kit/stem cell factor receptor-induced activation of phosphatidylinositol 3'-kinase is essential for male fertility. *Nat Genet* **24**, 157-162 (2000).
172. Lin, S.R., Yu, I.S., Huang, P.H., Tsai, C.W. & Lin, S.W. Chimeric mice with disruption of the gene coding for phosphatidylinositol glycan class A (Pig-a) were defective in embryogenesis and spermatogenesis. *Br J Haematol* **110**, 682-93 (2000).
173. Li, S. et al. Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene *pit-1*. *Nature* **347**, 528-33 (1990).
174. Laing, M.A. et al. Male sexual dysfunction in mice bearing targeted mutant alleles of the *PEA3* ets gene. *Mol Cell Biol* **20**, 9337-45 (2000).
175. Baker, S.M. et al. Male mice defective in the DNA mismatch repair gene *PMS2* exhibit abnormal chromosome synapsis in meiosis. *Cell* **82**, 309-19 (1995).
176. Gurtu, V.E. et al. Maternal effect for DNA mismatch repair in the mouse. *Genetics* **160**, 271-7 (2002).
177. Lydon, J.P. et al. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes and Development* **9**, 2266-2278 (1995).
178. Horseman, N.D. et al. Defective mammaryogenesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. *EMBO Journal* **16**, 6926-6935 (1997).
179. Ormandy, C.J. et al. Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. *Genes and Development* **11**, 167-178 (1997).
180. Lucas, B.K., Ormandy, C.J., Binart, N., Bridges, R.S. & Kelly, P.A. Null mutation of the prolactin receptor gene produces a defect in maternal behavior. *Endocrinology* **139**, 4102-7 (1998).
181. Sornson, M.W. et al. Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. *Nature* **384**, 327-33 (1996).
182. Kennedy, C.R.J. et al. Salt-sensitive hypertension and reduced fertility in mice lacking the prostaglandin EP<sub>1</sub> receptor. *Nature Medicine* **5**, 217-220 (1999).

183. Tilley, S.L. et al. Reproductive failure and reduced blood pressure in mice lacking the EP2 prostaglandin E2 receptor. *The Journal of Clinical Investigation* **103**, 1539-1545 (1999).
184. Hizaki, H. et al. Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP(2). *Proc Natl Acad Sci USA* **96**, 10501-10506 (1999).
185. Sugimoto, Y. et al. Failure of parturition in mice lacking the prostaglandin F receptor. *Science* **277**, 681-683 (1997).
186. Cho, C. et al. Haploinsufficiency of protamine-1 or -2 causes infertility in mice. *Nat Genet* **28**, 82-6 (2001).
187. Murer, V. et al. Male fertility defects in mice lacking the serine protease inhibitor protease nexin-1. *Proc Natl Acad Sci* **98**, 3029-33 (2001).
188. Skalhogg, B.S. et al. Mutation of the Calpha Subunit of PKA Leads to Growth Retardation and Sperm Dysfunction. *Mol Endocrinol* **16**, 630-9 (2002).
189. Varmuza, S. et al. Spermiogenesis is impaired in mice bearing a targeted mutation in the protein phosphatase 1gamma gene. *Dev Biol* **205**, 98-110 (1999).
190. Mani, S.K. et al. Requirement for DARPP-32 in progesterone-facilitated sexual receptivity in female rats and mice. *Science* **287**, 1053-6 (2000).
191. Osada, T., Watanabe, G., Sakaki, Y. & Takouchi, T. Puromycin-sensitive aminopeptidase is essential for the maternal recognition of pregnancy in mice. *Mol Endocrinology* **15**, 882-93 (2001).
192. Lufkin, T. et al. High postnatal lethality and testis degeneration in retinoic acid receptor alpha mutant mice. *Proc Natl Acad Sci U S A* **90**, 7225-9 (1993).
193. Lohnes, D. et al. Function of retinoic acid receptor gamma in the mouse. *Cell* **73**, 643-58 (1993).
194. Kastner, P. et al. Abnormal spermatogenesis in RXR beta mutant mice. *Genes Dev* **10**, 80-92 (1996).
195. Yeung, C.H., Sonnonberg-Riethmacher, H. & Cooper, T.G. Infertile spermatozoa of c-ros tyrosine kinase receptor knockout mice show flagellar angulation and maturational defects in cell volume regulatory mechanisms. *Biol Reprod* **61**, 1062-1069 (1999).
196. Yeung, C.H., Wagenfeld, A., Nieschlag, E. & Cooper, T.G. The cause of infertility of male c-ros tyrosine kinase receptor knockout mice. *Biol Reprod* **63**, 612-8 (2000).
197. Rigotti, A. et al. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc Natl Acad Sci USA* **94**, 12610-12615 (1997).
198. Trigatti, B. et al. Influence of the high density lipoprotein receptor SR-BI on reproductive and cardiovascular pathophysiology. *Proc Natl Acad Sci USA* **96**, 9322-9327 (1999).
199. Miettinen, H.E., Rayburn, H. & Krieger, M. Abnormal lipoprotein metabolism and reversible female infertility in HDL receptor (SR-BI)-deficient mice. *J Clin Invest* **108**, 1717-22 (2001).
200. Uhrin, P. et al. Disruption of the protein C inhibitor gene results in impaired spermatogenesis and male infertility. *J Clin Invest* **106**, 1531-9 (2000).
201. Ohtsuka, S. et al. SH2-B is required for both male and female reproduction. *Mol Cell Biol* **22**, 3066-77 (2002).
202. Tremblay, K.D., Dunn, N.R. & Robertson, E.J. Mouse embryos lacking Smad1 signals display defects in extra-embryonic tissues and germ cell formation. *Development* **128**, 3609-21 (2001).
203. Chang, H. & Matzuk, M.M. Smad5 is required for mouse primordial germ cell development. *Mech Dev* **104**, 61-7 (2001).
204. Supp, D.M., Witte, D.P., Bransford, W.W., Smith, E.P. & Potter, S.S. Sp4, a member of the Sp1-family of zinc finger transcription factors, is required for normal murine growth, viability, and male fertility. *Dev Biol* **176**, 284-299 (1996).
205. Zheng, Y., Deng, X., Zhao, Y., Zhang, H. & Martin-DeLeon, P. Spam1 (PH-20) mutations and sperm dysfunction in mice with the Rb(6.16) or Rb(6.15) translocation. *Mamm Genome* **12**, 822-9 (2001).
206. Pearce, R.V. et al. Reduced fertility in mice deficient for the POU protein sperm-1. *Proc Natl Acad Sci U S A* **94**, 7555-60 (1997).

207. Nayernia, K. et al. Asthenozoospermia in mice with targeted deletion of the sperm mitochondrion-associated cysteine-rich protein (Smcp) gene. *Mol Cell Biol* 22, 3046-52 (2002).
208. Pires-daSilva, A. et al. Mice deficient for spermatid perinuclear RNA-binding protein show neurologic, spermatogenic, and sperm morphological abnormalities. *Dev Biol* 233, 319-28 (2001).
209. Baudat, F., Manova, K., Yuen, J.P., Jasin, M. & Keeney, S. Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. *Mol Cell* 6, 989-98 (2000).
210. Romanienko, P.J. & Camerini-Otero, R.D. The mouse Spo11 gene is required for meiotic chromosome synapsis. *Mol Cell* 6, 975-87 (2000).
211. Mahendroo, M.S., Cala, K.M. & Russell, D.W. 5 $\alpha$ -reduced androgens play a key role in murine parturition. *Molecular Endocrinology* 10, 380-392 (1996).
212. Mahendroo, M.S., Cala, K.M., Landrum, C.P. & Russell, D.W. Fetal death in mice lacking 5 $\alpha$ -reductase type I caused by estrogen excess. *Molecular Endocrinology* 11, 1-11 (1997).
213. Caron, K.M. et al. Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipid adrenal hyperplasia. *Proc Natl Acad Sci USA* 94, 11540-11545 (1997).
214. Wishart, M.J. & Dixon, J.E. The archetype STYX/dead-phosphatase complexes with a spermatid mRNA-binding protein and is essential for normal sperm production. *Proc Natl Acad Sci U S A* 99, 2112-7 (2002).
215. Matzuk, M.M., Dionne, I., Guo, Q., Kumar, T.R. & Lebovitz, R.M. Ovarian function in superoxide dismutase 1 and 2 knockout mice. *Endocrinology* 139, 4008-4011 (1998).
216. Ito, Y.-S. et al. Reduced fertility in female mice lacking copper-zinc superoxide dismutase. *Journal of Biological Chemistry* 273, 7765-7769 (1998).
217. Yuan, L. et al. The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. *Mol Cell* 5, 73-83 (2000).
218. Yuan, L. et al. Female germ cell aneuploidy and embryo death in mice lacking the meiosis-specific protein SCP3. *Science* 296, 1115-8 (2002).
219. Freiman, R.N. et al. Requirement of tissue-selective TBP-associated factor TAF<sub>II</sub>105 in ovarian development. *Science* 293, 2084-7 (2001).
220. Zhang, D., Penttila, T.L., Morris, P.J., Teichmann, M. & Roeder, R.G. Spermiogenesis deficiency in mice lacking the Trf2 gene. *Science* 292, 1153-5 (2001).
221. Martianov, I. et al. Late arrest of spermiogenesis and germ cell apoptosis in mice lacking the TBP-like TLF/TRF2 gene. *Mol Cell* 7, 509-15 (2001).
222. Lee, H.-W. et al. Essential role of mouse telomerase in highly proliferative organs. *Nature* 392, 569-577 (1998).
223. Yanaka, N. et al. Insertional mutation of the murine kislmo locus caused a defect in spermatogenesis. *J Biol Chem* 275, 14791-4 (2000).
224. Jiang, J.Y., Imai, Y., Umez, M. & Sato, E. Characteristics of infertility in female hypothyroid (hyl) mice. *Reproduction* 122, 695-700 (2001).
225. Beck, A.R.P., Miller, I.J., Anderson, P. & Streuli, M. RNA-binding protein TIAR is essential for primordial germ cell development. *Proc. Natl. Acad. Sci. USA* 95, 2331-2336 (1998).
226. Yu, Y.E. et al. Abnormal spermatogenesis and reduced fertility in transition nuclear protein 1-deficient mice. *Proc Natl Acad Sci USA* 97, 4683-4688 (2000).
227. Zhao, M. et al. Targeted disruption of the transition protein 2 gene affects sperm chromatin structure and reduces fertility in mice. *Mol Cell Biol* 21, 7243-55 (2001).
228. Roby, K.F., Son, D.S. & Terranova, P.F. Alterations of events related to ovarian function in tumor necrosis factor receptor type I knockout mice. *Biol Reprod* 61, 1616-21 (1999).
229. Roest, H.P. et al. Inactivation of the HR23B ubiquitin-conjugating DNA repair enzyme in mice causes male sterility associated with chromatin modification. *Cell* 86, 799-810 (1996).
230. Ng, J.M. et al. Developmental defects and male sterility in mice lacking the ubiquitin-like DNA repair gene mHR23B. *Mol Cell Biol* 22, 1233-45 (2002).

231. Smith, C.L. et al. Genetic ablation of the steroid receptor coactivator-ubiquitin ligase, E6-AP, results in tissue-selective steroid hormone resistance and defects in reproduction. *Mol Cell Biol* 22, 525-35 (2002).
232. Dickins, R.A. et al. The ubiquitin ligase component Siah1a is required for completion of meiosis I in male mice. *Mol Cell Biol* 22, 2294-303 (2002).
233. Tanaka, S.S. et al. The mouse homolog of *Drosophila* Vasa is required for the development of male germ cells. *Genes Dev* 14, 841-853 (2000).
234. Kinuta, K. et al. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology* 141, 1317-24 (2000).
235. Johnson, L. & DeLuca, H. Vitamin D receptor null mutant mice fed high levels of calcium are fertile. *J Nutr* 131, 1787-91 (2001).
236. Sampson, M.J. et al. Immotile sperm and infertility in mice lacking mitochondrial voltage-dependent anion channel type 3. *J Biol Chem* 276, 39206-12 (2001).
237. Kreidberg, J.A. et al. WT-1 is required for early kidney development. *Cell* 74, 679-691 (1993).
238. Choi, J. et al. Mice deficient for the wild-type p53-induced phosphatase gene (*Wip1*) exhibit defects in reproductive organs, immune function and cell cycle control. *Mol Cell Biol* 22, 1094-105 (2002).
239. Vainio, S., Heikkila, M., Kispert, A., Chin, N. & McMahon, A.P. Female development in mammals is regulated by Wnt-4 signalling. *Nature* 397, 405-409 (1999).
240. Parr, B.A. & McMahon, A.P. Sexually dimorphic development of the mammalian reproductive tract requires *Wnt-7a*. *Nature* 395, 707-710 (1998).
241. Luoh, S.-W. et al. *Zfx* mutation results in small animal size and reduced germ cell number in male and female mice. *Development* 124, 2275-2284 (1997).
242. Rankin, T., Talbot, P., Lee, E. & Dean, J. Abnormal zonae pellucidae in mice lacking *ZP1* result in early embryonic loss. *Development* 126, 3847-3855 (1999).
243. Rankin, T.L. et al. Defective zonae pellucidae in *Zp2*-null mice disrupt folliculogenesis, fertility and development. *Development* 128, 1119-26 (2001).
244. Rankin, T. et al. Mice homozygous for an insertional mutation in the *Zp3* gene lack a zona pellucida and are infertile. *Development* 122, 2903-10 (1996).
245. Liu, C. et al. Targeted disruption of the *mZP3* gene results in production of eggs lacking a zona pellucida and infertility in male mice. *Proceedings of the National Academy of Sciences USA* 93, 5431-5436 (1996).